Evaluating Fungicide Timing for Leaf Spot Diseases of Wheat and Fungicide Resistance in *Pyrenophora tritici-repentis*

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ABSTRACT

Fungal diseases are a major issue in wheat growing areas of the prairies and can result in significant yield losses if left unchecked. Some of the most common diseases affecting wheat are leaf spots, caused by several pathogen species that make up the leaf spotting complex, including tan spot of wheat, caused by *Pyrenophora tritici-repentis*. The optimal timing to apply fungicide to protect wheat against leaf spots is during the flag leaf stage (ZGS39), however, another disease, fusarium head blight (FHB), is also a major issue in wheat grown in the prairies and is capable of devastating an entire crop. The optimal timing to apply fungicide to control FHB is during anthesis (ZGS60). It is also possible for pathogen species to become insensitive to fungicides over time if they are frequently exposed to the same class of fungicide, especially site specific fungicides such as quinone outside inhibitors (QoI) like pyraclostrobin or demethylation inhibitors (DMI) like propiconazole. It is therefore imperative to limit the amount of fungicide applied to fields to reduce the exposure to pathogens that may lead to insensitivity.

The objective of this project was to evaluate the efficacy of applying synthetic fungicides and a biofungicide at two times, flag leaf emergence and anthesis, to determine if application at either of these timings would adequately control leaf spots. Five fungicide products or product combinations were applied at two growth stages, as single or double applications at five locations in 2013 and 2014 using the wheat cultivar Carberry. The results showed that applying fungicide at anthesis stage provided adequate control of leaf spots compared to applying at flag leaf stage. Although applying fungicide at anthesis stage resulted in a higher incidence of leaf spots when disease pressure was high, yield was the same whether fungicide was applied at flag leaf stage or anthesis stage.

The second objective of this study was to determine the baseline sensitivity of the *Pyrenophora tritici-repentis* populations from Alberta and Saskatchewan to two fungicide modes of action, QoIs and DMIs, on spore germination and radial growth on fungicide amended solid agar media. Seventy-one isolates were collected from Saskatchewan in 2013-2014 and AB in 2010 and radial growth measured on V8-PDA media amended with propiconazole, pyraclostrobin and pyraclostrobin with SHAM at five different concentrations. Spore germination was also determined by counting the number of germinated spores in a 5 µL mL^{-1} spore-water suspension treated with 10 µL of 10x diluted lactophenol. The effective concentration to reduce spore germination by 50% (EC_{50}) values were determined by calculating the number of spores that successfully germinated against the total number of spores. There were also five different fungicide concentrations for the spore germination treatments. The results of the study created a baseline for pyraclostrobin and propiconazole sensitivity and it was determined that QoI and DMI insensitivity has not occurred in the *P. tritici-repentis* population in Saskatchewan and Alberta.

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LIST OF ABBREVIATIONS

CWRS	Canadian western red spring
CWAD	Canadian western amber durum
CPS	Canadian prairie spring
CWES	Canadian western extra strong
CWSWS	Canadian western soft white spring
FHB	Fusarium head blight
DON	Deoxynivalenol
IDM	Integrated disease management
ZGS	Zadoks growth stage
HRWW	Hard red winter wheat
SRWW	Soft red winter wheat
DMI	Demethylation inhibitor
FRAC	Fungicide Resistance Action Committee
AUDPC	Area under disease progress curve
QoI	Quinone outside inhibitor
$ED_{90}S$	Effective dose at which 90% of spore germination is inhibited
Ptr	*Pyrenophora tritici-repentis*
TKW	Thousand kernel weight
TW	Test weight
LB	Leaf blotch
FDK	Fusarium damaged kernels
EC_{50}	50% of maximum effective concentration
PPM	Parts per million
PDA	Potato dextrose agar
SHAM	Salicylhydroxamic acid
ANCOVA	Analysis of covariance

CHAPTER 1

1. Introduction

Wheat (*Triticum aestivum* L. subsp. *aestivum)* is among the most important crops grown in the province of Saskatchewan, with 915 million tonnes harvested from 8.2 million acres in 2014. One of the major issues with cultivating wheat is yield loss brought on by disease pressure. Due to changes in cultural practices, such as the advent of no-till farming, many pathogens that rely on crop residue to complete their lifecycle, such as the pathogens in the leaf-spotting complex, have increased in occurrence. Breeding for leaf spot resistance in wheat has not been a priority as leaf spots are not considered a major issue, with less effect on yield than the rusts and fusarium head blight (*Fusarium graminearum* Schwabe, group II [anamorph]) (FHB). However, leaf spots are capable of causing a 50% decrease in yield when severity is high. The issue faced by growers is that applying fungicide at flag leaf stage (Zadoks growth stage (ZGS) 39) alone may leave the crop susceptible to FHB, as the optimal timing to apply fungicide to control FHB is believed to be at anthesis (ZGS60).

Applying fungicide at both ZGS 39 and 60, which are usually within 5-10 days of each other in Saskatchewan, is inadvisable because of the added cost and the probability of pathogens developing reduced sensitivity to fungicide. Determining whether applying fungicide at anthesis stage (ZGS60) would adequately control leaf spots would be beneficial as it would reduce costs and labor for growers while at the same time reducing pathogen exposure to fungicide and the risk of insensitivity developing.

The two most commonly used fungicide groups to control leaf spots, quinone outside inhibitors (QoIs) and demethylation inhibitors (DMIs), are single-site inhibitors and insensitivity in pathogen species may develop rapidly because of overexposure. This has been documented in

a number of pathogen species in the United States and across Europe. Two mutations, G143A and F129L are believed to be responsible for insensitivity to QoI fungicides in many of these pathogens.

Pyrenophora tritici-repentis (Died.) Drechsler (anamorph *Drechslera tritici-repentis*) is the most common leaf spotting pathogen species of wheat in Saskatchewan. Research has not been conducted in the province to determine if insensitivity to either QoI or DMI fungicides has occurred in the pathogen population. Determining a baseline for sensitivity to these fungicides in *P. tritici-repentis* would be beneficial to determine whether insensitivity has already occurred in the pathogen population and to confirm insensitivity if it occurs in the future.

There were two hypotheses for this study:

1. Wheat leaf disease severity and productivity varies based on fungicide application timing, as well as fungicide mode of action.

2. Sensitivity to each fungicide mode of action varies among isolates of *P. tritici-repentis.*

The objectives that follow from the hypotheses are:

1. To determine whether applying fungicide at anthesis stage (ZGS60) would adequately control leaf spots compared to applying fungicide at flag leaf stage (ZGS 39). Determining whether applying fungicide at anthesis stage, the recommended timing to control FHB, would also adequately control leaf spots would be beneficial to growers. It would allow them to apply fungicide at only one timing rather than two to control leaf spotting diseases of spring wheat, thereby gaining an economic benefit while at the same time reducing the risk of development of fungicide insensitive pathogens due to overexposure.

2. The objective of this study was to determine a baseline sensitivity for isolates of *Pyrenophora tritici-repentis* collected in Saskatchewan and Alberta to propiconazole and

pyraclostrobin. This would allow us to determine if reduced sensitivity had occurred based on the EC_{50} values of the isolates, as well as functioning as a baseline to determine if reduced sensitivity to these fungicides occurs in the future.

CHAPTER 2

2. Literature Review

2.1 Wheat

Wheat (*Triticum aestivum* L. subsp. *aestivum*) is one of the most important food crops in the world, being a staple for over 35% of the population. It is expected that the demand for wheat will increase exponentially, from 721 million tonnes in 2015 to an estimated 900 million tonnes in 2050 (FAO, 2015; Dixon et al., 2009). Spring wheat is grown on nearly 200 million acres worldwide (Bockus et al., 2001); production in Canada in 2014 was 21.2 million tonnes, 9.15 million tonnes of which were harvested in the province of Saskatchewan from 8.2 million acres (StatsCan, 2014). Over 96% of wheat produced in Canada is spring wheat (DePauw and Hunt, 2001). There are five quality classes of spring wheat produced in western Canada that include: Canadian western red spring (CWRS), Canadian western amber durum (CWAD), Canadian prairie spring (CPS), Canadian western extra strong (CWES), and Canadian western soft white spring (CWSWS). CWRS is the most commonly grown class in western Canada, accounting for 60-72% of wheat produced (Lamari et al., 2005).

Wheat production must overcome a number of obstacles to ensure continued yield increases, including abiotic factors such as those associated with climate change, as well as biotic factors, including insects and diseases. In a large number of wheat growing areas of the world, there has been a noticeable increase in foliar leaf spot disease severity, owing mainly to changes in cultural practices, such as the adoption of no-till in addition to the replacement of taller cultivars with high-yielding semi-dwarf varieties (Eyal et al., 1987; Chaurasia et al., 1999).

2.2 Leaf spotting diseases of wheat and impact on yield

2.2.1 Tan Spot disease

Fungi are the number one cause of plant diseases and have a significant effect on wheat production (McGrath, 2004). *Pyrenophora tritici-repentis* (Died.) Drechsler (anamorph *Drechslera tritici-repentis;* DTR) is an ascomycete from the class Dothideomycetes that causes tan spot, a leaf disease of wheat (Gilbert and Woods, 2001). Tan spot is a major disease of wheat on the Canadian prairies as well as several other wheat-producing countries throughout the world. Tan spot was first reported in the Canadian prairies in the 1930's and regularly reported as a serious problem for wheat growers annually by the 1970s (Tekauz 1976). Tan spot of wheat has recently become the most important disease in the leaf spot complex occurring in western Canada; it results in significant yield loss and reduced quality of grain (Gilbert and Woods, 2001).

Tan spot disease begins on wheat leaves after ascospores infection, which are produced in pseudothecia (sexual fruiting bodies); the majority of the spores are dispersed within a few metres of the infested wheat stubble and germinate on leaves under wet conditions (Rees and Platz, 1980; Rees and Platz, 1988). The pseudothecia usually mature by early spring, however, many factors play a part in the time required for pseudothecial development, including host genotype and environmental and temporal conditions of the pathosystem (Moreno et al., 2012). As a result of western Canada's low temperature, mature pseudothecia are usually observed on two-year-old stubble (Bailey et al., 2001). Crop residue is considered the main source of inoculum, however, the pathogen also has numerous alternative hosts, including many wild grass species that may act as reservoirs of inoculum, preserving genetic variants and enabling inoculum to survive between wheat growing seasons (Moreno et al., 2012). *Pyrenophora tritici-repentis* penetrates the leaf epidermis by punching a hole through it with its appresoria, specialized structures of the germ tube

and continues to develop and infect the plant through the intercellular spaces of the mesophyll (Loughman and Deverall, 1986). Ascospores, the sexual spores of the fungus, are often the primary inoculum. Successful pathogen infection is followed by repeated cycles of conidial production, the asexual spores (Howard and Morrall, 1975). Tan spot symptoms begin as tan-brown flecks that expand into larger tan coloured blotches surrounded by a yellow border; as disease severity progresses, the lesions coalesce and the pathogen produces conidiophores and conidiospores causing the centre of the lesion to darken significantly and appear black.

2.2.2 Leaf spotting diseases

There are a variety of other leaf spot diseases that affect spring wheat, including those pathogen species that make up the septoria leaf spot complex. These include *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), the causal agent of septoria leaf blotch, as well as *Phaeosphaeria nodorum* (anamorph: *Stagonospora nodorum*) and *Phaeosphaeria avenaria* (anamorph: *Stagonospora avenae*), the causal agents of stagonospora blotch (Goodwin, 2012). The diseases caused by these pathogens are often very difficult to distinguish in the field (Goodwin, 2012). Foliar leaf spot diseases of wheat commonly occur in the Great Plains area of the United States and Canada (Murray et al., 1998). S*tagonospora avenae* is less prevalent in fields compared to the other two pathogens, except when conditions are very warm and very dry (Ponomarenko et al., 2011). Stagonospora blotch however, is most apparent in fields during warm and dry conditions (Ponomarenko et al., 2011). The diseases that comprise the septoria leaf spot complex are often not the most prevalent diseases found in wheat fields in North America, as most often rust, fusarium head blight and spot blotch are more severe (Goodwin, 2012).

Research by Sutton and Vyn (1990) suggested that *P. tritici-repentis* infection supresses *Septoria tritici* infection, especially in fields where wheat residue is abundant. *Pyrenophora tritici-repentis* was also seen to out-compete *Stagonospora nodorum* in test plots of wheat (Adee et al., 1990). *Pyrenophora tritici-repentis* has a much shorter latent period of 5-8 days (Riaz et al., 1991) compared to the 3-4-week latent period of *Septoria tritici* (Shaw, 1990), so it is likely that tan spot would out-compete septoria leaf blotch as well.

Surveys conducted by Sutton and Vyn (1990), as well as Wolf and Hoffmann (1995), confirmed that the most severe outbreaks of leaf spot diseases in spring wheat occurs in fields managed with non-inversion tillage. Non-inversion tillage is used to break up hard pans and increase the available soil depth by making thin vertical slices in the soil. The soil is lifted and dropped which causes shattering on either side of the cut slot creating numerous cracks, which allows the soil to be penetrated by water, gases and roots thereby making deeper layers accessible beneath the hard pan. It was also reported that cold, dry conditions during the autumn and winter months increased the probability of pseudothecia survival (Summerell and Burgess, 1989), while warm, wet conditions resulted in the breakdown of crop debris, limiting the number of pseudothecia in the spring (Stott et al., 1986).

2.3 Fungicide Control of wheat diseases

Tan spot is capable of causing up to 50% yield loss in wheat, depending on management practices and environmental factors (Hosford, 1971; Hosford and Busch, 1974; Rees et al., 1982; Rees and Platz, 1983; Tekauz and Platford, 1982); however, losses as high as 75% have been recorded (Rees et al., 1981). Yield loss due to septoria leaf blotch may be as high as 30-50% during

severe epidemics, but is often much lower (Eyal et al., 1987). Yield loss due to stagonospora blotch is often much lower, reaching 15% during severe epidemics (King et al., 1983).

Research has demonstrated that yield loss caused by foliar leaf spot diseases may vary greatly due to environmental conditions and cultural practices. Rees et al. (1982) reported that in Australia, tan spot reduced yield in control plots by an average of 49%. Evans et al. (1999) found that in Oklahoma, tan spot reduced yields by 15% in inoculated plots compared to plots treated with fungicide. Villareal et al. (1995) reported 43% lower yields in untreated plots in Mexico compared to those treated with fungicide. De Milliano and Zadoks (1985) observed a 38% yield loss in African wheat cultivars exposed to foliar leaf spot diseases in growth chamber studies. Lapis (1985) discussed that foliar leaf spot diseases resulted in a yield loss of 40% in the Philippines, while Raemakers (1988) found that yield was reduced by 85% in infected wheat plots in Zambia. Yield losses of 19% and 43% were reported in wheat cultivars grown in Brazil and Nepal, respectively (Da Luz, 1984; Sharma and Duveiller, 2006). Research by Shabeer and Bockus (1988) in Kansas suggested that 17% of the total yield loss observed in winter wheat was due to early season tan spot infection, while 50% of the total observed yield loss had occurred by the boot stage. Rees and Platz (1983) however, determined that in Australia, yield was reduced by 13% due to disease that had occurred at flag leaf stage or earlier and 35% by disease that had occurred after flag leaf stage with an overall yield reduction of 48% in wheat cultivar Banks.

Diseases associated with seed infection, such as red-smudge and black point, cause grain shriveling (Fernandez et al., 1994), which has been shown to reduce yield, kernel weight (Schilder and Bergstrom, 1990; Shabeer and Bockus, 1988), kernels per head (Schilder and Bergstrom, 1990), total biomass (Kremer and Hoffmann, 1992), and grain quality.

Fusarium head blight (FHB) (*Fusarium graminearum* Schwabe, group II [anamorph]) was a major issue in the Pacific North West of the US as well as the Canadian province of Manitoba as early as 1993. At the time, fungicides were considered the only means of defense against this aggressive disease and a way to control outbreaks until genetically resistant varieties of spring wheat could be developed (McMullen et al., 1997a). Fusarium head blight causes either the entire spike or a portion of the spike to undergo premature senescence, which results in reduced yield and grain quality (Haidukowski et al., 2005). Yield losses may reach 70% during years in which FHB infection is severe. Under favourable environmental conditions, *F. graminearum* can produce several mycotoxins, the most common of which is deoxynivalenol (DON) (Bottalico and Perrone, 2002). DON may be present in food and feed from infected grain and can result in neurotoxic and immunotoxic effects in mammals including humans and livestock (FAO/WHO, 2001). Several European countries have placed limits on the levels of DON allowable in wheat and cereal products intended for human consumption, 2.0 mg kg^{-1} for wheat and unprocessed wheat products, 1.0 mg kg^{-1} for wheat flour, pasta and bakery products, and 0.5 mg kg^{-1} for food products intended for infants and young children (Koornneef et al., 2002). The allowable DON limits in Canadian wheat are 2.0 mg kg^{-1} for uncleaned soft wheat for use in non-staple foods and 1.0 mg kg^{-1} for uncleaned soft wheat for use in baby foods. There is conflicting evidence on the effect of fungicides to reduce mycotoxin production in wheat due to *F. graminearum* (Haidukowski et al., 2005). There are reports that certain triazole fungicides, alone or in combination, are capable of reducing FHB severity and DON production, however other reports suggest that azoxystrobin may actually increase DON production, although it may reduce the amount of Fusarium damaged kernels (Edwards et al., 2001).

The decision to apply fungicide to a crop is largely dependent on the cost of fungicide and the price of wheat. To ensure fungicide effectiveness, application must be made prior to the appearance of disease symptoms. Positive returns are more likely if wheat prices are high relative to fungicide costs. Cost to benefit ratios must be considered when applying fungicide, as low wheat prices and/or high application costs may negate any benefit of fungicide even when disease levels are high (Wiik and Rosenqvist, 2010; Wegulo et al., 2011).

2.3.1 Timing of Application

Under current integrated disease management (IDM) guidelines, fungicide application for leaf spot diseases are based on disease level and plant growth stage and are recommended for use only on susceptible cultivars (Bowden, 1995; Heger et al., 2003; Paveley et al., 1997). Fungicide application is often used to protect the flag leaf from pathogen infection (Wegulo et al., 2012). The majority of fungicides are protective in nature, with very few effective after disease symptoms appear on plants (McGrath, 2004). Protection of the flag leaf in wheat is paramount, as flag leaf health is a determining factor in kernel development, grain filling (weight) and yield (Ruske et al., 2003; Simpson, 1968). A second fungicide application at flowering is made if FHB is expected to be a problem. Fungicide should be applied at flag leaf emergence to control leaf spots; application before flag leaf emergence leads to reduced disease control of upper leaves and lower yield (DeWolf et al., 2012). The optimal time of fungicide application in wheat has been largely inconclusive, although several studies have suggested that application at flag leaf stage improves yield (Wegulo et al., 2012).

Before 1997, Syngenta Crop Protection recommended application of propiconazole at the flag leaf stage (ZGS39), to most effectively suppress leaf spot diseases; however, the Environmental Protection Agency (EPA) did not allow application of propiconazole during this

period (Wiersma and Mottenberg, 2005). The optimal timing to control fusarium head blight was reported by Halley et al. (2001) to be at the beginning of anthesis, ZGS60. Optimum application timing of fungicides to control tan spot and other leaf diseases of spring wheat is believed to be at ZGS39 (Wiersma and Motteberg, 2005).

Wiersma and Motteberg (2005) noted that applying half the labelled rate of propiconazole with trifloxystrobin at ZGS15 (seedling stage) in addition to applying tebuconazole at ZGS60 proved the most effective in controlling leaf spot diseases as well as providing the largest gain in grain yield and the greatest improvement in grain quality. They discussed how the combination of propiconazole with trifloxystrobin at ZGS15 and tebuconazole at ZGS60 increased grain yield by 11-31% between 2001 and 2003. Hunger and Brown (1987) wrote that *P. tritici-repentis* isolates were least sensitive to mycobutanil, moderately sensitive to tebuconazole, and highly sensitive to propiconazole based on samples collected in the USA. Jørgensen and Thygesen (2006) found that pyraclostrobin, picoxystrobin, propiconazole and prothioconazole were the most effective fungicides for control of tan spot.

Turkington et al. (2015) tested the efficacy of half-rates of fungicide with herbicide, as well as full-rates, on leaf disease when applied at the two-to-three leaf stage. They found that applying a half-rate or full-rate of fungicide at the two-to-three leaf stage did not adequately control leaf disease on the flag or penultimate leaves. However, they stated that there was no significant difference between split applications of fungicide at the two-to-three leaf stage and again at the flag leaf stage (full or half-rates) and a full-rate of fungicide at the flag leaf stage. Both treatments adequately controlled leaf spotting diseases.

The combination of tebuconazole and prothioconazole is recommended to reduce FHB incidence and DON levels as they have proven to be more effective than tebuconazole alone (Paul

et al., 2007). Fungicide application studies were also conducted by Martin and Johnson (1982), who found that FHB was reduced by the addition of propiconazole resulting in a 34% increase in yield when applied during the flowering stage. Similar tests were also conducted by Boyacioglu et al. (1992), who found that FHB severity was reduced by 39-61% through application of triadimefon and propiconazole at flowering stage. Additionally, experiments conducted in both Minnesota and North Dakota discovered that tebuconazole was the most effective of the triazole fungicides, reducing FHB severity by 71% when applied at flowering stage (McMullen et al., 1997a; McMullen, 1998). Ferhman and Ahrens (1984) noted that yield losses were reduced by 36-48% after two applications of prochloraz in trials artificially inoculated with *F. graminearum*. McMullen et al. (1997b) saw that in field trials conducted in Minnesota and North Dakota, tebuconazole reduced FHB severity by 71% and was the most consistent among triazole fungicides at reducing FHB incidence.

Bowden (1995) suggested that yield response to fungicide application could be calculated based on foliar disease potential, stating that when disease was low, medium or high, fungicide application would correspondingly increase yield by 5%, 10%, and 15% based on foliar fungicide trials on hard red winter wheat (HRWW) in Kansas. Ransom and McMullen (2008), as well as Thompson et al. (2014), found that yields increased in HRWW in response to fungicide application when disease pressure was high in North Dakota and Oklahoma, respectively. Lopez et al. (2015) and Wegulo et al. (2012), however, noted very little to no yield response to fungicide when disease pressure was low in Texas and Nebraska, respectively. Weisz et al. (2011) demonstrated on soft red winter wheat (SRWW) in the mid-Atlantic region of the US that environments with average disease levels did not respond with yield increases great enough to justify foliar fungicide application from an economic standpoint. Bergstrom (2010) wrote that on average, when disease

levels in general were high, fungicide-treated fields yielded up to 20% more compared to non-treated fields.

Applying fungicides may increase the production of plant antioxidants and slow chlorophyll and leaf protein degradation, thus preserving yield potential at the time of application (Zhang et al., 2010; Edwards et al., 2012). Some fungicides have been suggested to delay leaf senescence, allowing greater nutrient accumulation during later plant developmental stages (Morris et al., 1989; Dimmock and Gooding, 2002). De Wolf et al. (2012) and Kelley (2001), however, indicated the effect of foliar fungicides on yield in the absence of disease pressure is "highly variable" in winter wheat. This may be due to a number of factors including environmental conditions, timing of fungicide application, yield potential, cultivar disease resistance and the severity and incidence of different foliar diseases that may be present.

2.4 Pathogen Insensitivity

Currently wheat growers are highly dependent on the availability of effective cereal fungicides to maintain high yields. (Jørgensen, 2008). Fungicides first began to be used on cereal crops when systemic products were developed during the latter part of the 1960's (Hewitt, 1998). Intensive spraying of fungicides has led to the development of insensitivity over time to many fungicide groups including benzimidazols, DMI's, and strobilurins in diseases such as powdery mildew, scald (*Rhynchosporium secalis)* and net blotch (*Pyrenophora teres*) (Jørgensen, 2008). The most common fungicides used to control cereal diseases are triazoles, strobilurins, morpholines, carboxamides, and chlorothalonil (Jørgensen, 2008).

General principles to limit pathogen insensitivity to fungicides have been proposed by the Fungicide Resistance Action Committee (FRAC), who suggested: reducing the exposure of pathogens to fungicides, avoiding the use of fungicides when pathogen populations are already

well-established in a crop, alternating fungicides with different modes of action or mixing different fungicides to be applied, and using doses of the fungicide that are effective in killing the pathogen population as opposed to using multiple small doses that increase insensitive individuals in the population (FRAC 1998). Often these principles are redundant or difficult to follow but at the very least measures should be taken to limit the contact of fungicides with pathogen populations (Jørgensen, 2008).

It is essential that regular monitoring of pathogen populations be conducted to avoid fungicide insensitivity resulting in the loss of efficacy of important chemical groups (FRAC, 1998). Monitoring is especially critical for new chemical groups to determine baseline sensitivity, so that changes in pathogen sensitivity can be discovered as soon as they appear, as well as aiding in the development of proper anti-resistance management strategies (Bayles et al., 2001)

2.4.1 Types of Insensitivity

There are two types of insensitivity that generally occur from the continual interaction of fungicides with plant pathogens. The first involves a sudden loss of effectiveness that occurs because two distinct populations of plant pathogens have developed: those populations that are sensitive to the fungicide and those that have become insensitive. This type of insensitivity is known as qualitative, single-step, discrete, disruptive, or discontinuous insensitivity, and often results in permanent insensitivity of the pathogen population even if fungicide use is reduced or halted entirely (Brent and Hollomon, 2007). The second type of insensitivity develops less suddenly, and is marked by a gradual decline in the efficacy of the fungicide on pathogen populations. This form of insensitivity is known as quantitative, multi-step, continuous, directional, or progressive insensitivity; fortunately, this form of insensitivity can be easily

reversed if fungicide use on the plant population is reduced or alternative fungicides with different modes of action are used on the plant population instead (Brent and Hollomon, 2007).

2.4.2 Triazole Sensitivity

Triazole fungicides are curative and systemic moving through the xylem of the plant (Wegulo et al., 2012). Triazoles slow the growth of the pathogen by inhibiting sterol biosynthesis (Buchnenaur, 1987). They are capable of redistribution within the plant and are effective against early fungal infection (Hewitt, 1998). Triazoles have been used to control cereal diseases for over 25 years, with significant use in Europe during the 1980's to control powdery mildew (Jørgensen, 2008). Registration of propiconazole to control powdery mildew of barley was retracted in 1996, because the disease developed widespread insensitivity to the fungicide (Jørgensen, 2008). Triazoles have also been used to control septoria leaf blotch in cereals since the 1990's; however, due to several point mutations in the pathogen population, the efficacy of many triazole fungicides such as epoxiconazole and prothioconazole have decreased significantly in several nations including France, The United Kingdom, and Denmark (Clark and Paveley, 2005; Jørgensen and Thygesen, 2006).

Gaurilcikiene and Ronis (2006) tested the efficacy of several strobilurin and triazole fungicides on stagonospora leaf blotch and tan spot of winter wheat between 2003 and 2004. They found that all the strobilurins and triazoles tested had similar efficacy against these leaf diseases, except propiconazole, which was significantly less effective and resulted in a much higher area under the disease progress curve (AUDPC). There have been numerous studies on fungicide sensitivity in *Pyrenophora teres,* the causal agent of net blotch of barley that have demonstrated variation in genetic sensitivity to fungicide as well as differences in the efficacy of various triazole

fungicides (Sheridan and Nendick, 1987; Peever and Milgroom, 1994; Locke, 1996, 2000; Campbell and Crouse, 2002).

2.4.3 Strobilurin Sensitivity

Strobilurins, or quinone outside inhibitors (QoI), function as respiration inhibitors, affecting the Quinone outside site of the mitochondrial gene, cytochrome *b*, located in complex III (Sierotzki et al., 2007). Strobilurin fungicides such as azoxystrobin and pyraclostrobin are preventative as well as protective, acting to inhibit spore germination and early infection, and are considered to be locally systemic (Butzen et al., 2005). Unlike other classes of fungicide, QoIs function by inhibiting a single target, cytochrome *bc1*, which is encoded by a mitochondrial gene, cyt *b*. There are several very important differences between nuclear and cytoplasmic inheritance. The segregation of nuclear encoded traits occurs during sexual recombination as a result of meiosis, whereas segregation of mitochondrial genes occurs as a result of mitotic cell division. Inheritance of nuclear genes is bi-parental, while inheritance of mitochondrial genes is uniparental, most often maternally inherited. The rearrangement of mitochondrial genomes has some effect on cell aging in animals but does not appear to affect metabolic processes, meaning fitness is not affected. It was reported in animal cells that the mutation rate was lower in nuclear DNA compared to mitochondrial DNA due to more effective repair mechanisms, however the opposite was observed in yeasts (Clark-Walker, 1992). Mitochondrial genomes are often much less genetically diverse, in comparison to nuclear genomes (Gisi et al., 2002). Early mitotic events including mutation rate and repair mechanisms as well as intracellular selection most likely play a part in the evolution of individual insensitivity to QoI fungicides. The evolution of population insensitivity to a QoI fungicide, however, is due to selection pressure of the fungicide on the population as well as recurrent mutation, recombination and migration of the pathogen (Gisi et al., 2002).

Due to concern with fungicide insensitivity, strobilurin use is limited in Canada and they should only be used when rotated with other fungicides (Chang et al., 2007). QoI products were first introduced into the cereal market in 1996 (Sierotzki et al., 2007). The first recorded incidence of QoI fungicide insensitivity was in wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) in northern Germany in 1998, two years after they were first introduced (Heaney et al., 2000). Barley powdery mildew (*B. graminis* f. sp. *hordei*) developed insensitivity in northern Germany by 1999 (Heaney et al., 2000). Reports of strobilurin insensitive septoria tritici blotch (*M. graminicola*) isolates in the UK and Ireland were made in 2002 (Fraaije et al., 2003). The first insensitive *P. tritici-repentis* isolates were reported in 2003 (FRAC, 2002). The first QoI insensitive isolates of *P. tritici-repentis* were detected in middle Sweden. By 2004, insensitivity in *P. tritici-repentis* was observed in field populations (Sierotzki et al., 2007).

Mutations occurring in the mitochondrial gene cytochrome *b* are responsible for insensitivity to QoI products that have been observed in *P. tritici-repentis* (Brasseur et al., 1996). There are several amino acid substitutions that have occurred in the pathogen population providing varying levels of insensitivity (Fisher and Meunier, 2001). The most common mutation is the substitution of glycine for alanine at amino acid position 143, and is subsequently referred to as G143A (Sierotzki et al., 2007). This particular substitution provides the greatest insensitivity to QoI products when compared with other such mutations. Another mutation, F129L, in which an amino-acid is changed from phenylalanine to leucine at position 129 was first reported in populations of *Pyricularia grisea* (Sacc) (Farman, 2001) and *Pythium aphanidermatum* (Edson) Fitzp (G Olaya, pers comm, 2001 in Sierotzki et al., 2007). The level of insensitivity conferred by F129L is lower than that provided by G143A. The F129L mutation was first detected in *P. tritici-repentis* but was rapidly overtaken by the G143 mutation in the population by 2004 and a third

mutation, in which glycine changes to arginine at amino acid position 137 was reported in 2007 (Sierotzki et al., 2007). The G143A mutation confers complete insensitivity, making QoI fungicides useless in controlling the disease (Gisi et al., 2002). The F129L and G137R mutations, on the other hand, confer only partial resistance, resulting in reduced sensitivity of the pathogen to QoI fungicides (Kim et al., 2003; Pasche et al., 2004, 2005; Sierotzki et al., 2007). F129L and G143A mutants experienced no significant negative effects on enzyme activity. As such, there were no recorded fitness penalties in individuals with these mutations (Brasseur et al., 1996). The G137R mutation however, had an effect on respiratory competence, which may lead to mutants that are unable to survive (Wise et al., 2009).

Five significant differences were detected when the amino-acid sequences of the *bc1* complex of wild-type fungal isolates of *Aspergillus nidulans* (Eidam) Winter and *Saccharomyces cerevisiae* Meyer ex. Hansen were compared to those of basidiomycetes that naturally produce strobilurins as metabolic by-products, such as *Strobilurus tenacellus* (Pers ex. Fr) Singer, *Mycena galopoda* and *M. viridimarginata*. These were: a change from threonine to isoleucine (T127I), from alanine to serine (A153S), from serine to glutamine (S255Q), from asparagine to aspartic acid (N262D), and the G143A mutation (Zheng and Köller, 1997). The changes in the amino-acid sequence of strobilurin producing basidiomycetes is thought to protect the fungus from self-poisoning (Wise et al., 2009). As the *bc1* complex of these fungi is functional, a fitness penalty cannot be associated with these amino-acid changes, at least in these specific fungi, or they would not survive (Wise et al., 2009). Wise et al. (2009) did however observe a fitness penalty with regards to growth rate when fungicide-adapted isolates, having been exposed to increasing levels of strobilurin fungicide, of *P. tritici-repentis* were exposed to non-amended agar plates, specifically strobilurin-adapted isolates. It may be possible that the fitness penalty observed with

isolates of *P. tritici-repentis* was due to the energy requirement of hyphal efflux transporters (Reimann and Deising, 2004).

2.4.4 Fungicide Insensitivity in *Pyrenophora tritici-repentis*

Although studies on fungicide insensitivity in *P. tritici-repentis* are limited, fungicide insensitivity in this pathogen has been noted (Anon., 2005). Reimann and Deising (2005) collected isolates of *P. tritici-repentis* from fields in the Saxony-Anhalt and Schleswig-Holstein regions of Germany and conducted spore germination assays using epoxiconazole and kresoxim-methyl. They recorded that isolates collected in Saxony-Anhalt showed no difference in sensitivity based on field fungicide history. The isolates collected in Schleswig-Holstein, however, had a history of repeated fungicide use and showed ED_{90}s 2-3 times higher than isolates collected in Saxony-Anhalt. Campbell and Crouse (2002) also found that sensitivity to a number of fungicides varied by field, however sensitivity to propiconazole and tebuconazole was similar. Insensitivity to QoI fungicides have been documented in a number of pathogens of various crops across Europe and Asia including wheat (*Blumeria graminis* f. sp. *tritici*) (Sierotzki et al., 2000), banana (*Mycosphaerella fijiensis*) (Sierotzki et al., 2000), grape (*Plasmopara viticola*) (Gisi et al., 2000), barley (*Blumeria graminis* f. sp. *hordei*) (Heaney et al., 2000) and apple (*Venturia inaequalis*) (Steinfield et al., 2002). Vincelli and Dixon (2001) performed in vitro sensitivity assays on *Pyricularia grisea* isolates collected from fungicide-treated ryegrass in Kentucky and Illinois and reported that isolates had become insensitive to QoI fungicides, having over 1,000-fold less sensitivity in comparison to baseline isolates that had never been exposed to fungicides.

Glasshouse experiments conducted on *P. tritici-repentis* showed that those isolates possessing the G143A mutation were not controlled with only QoI fungicides and required a second fungicide application similar to what had previously been reported on *M. graminicola*

(Fraajie et al., 2005). However, isolates possessing the F129L mutation were still adequately controlled with only the QoI fungicide application (Fraajie et al., 2005). It was reported that the effects of QoI fungicides are severely reduced with high frequencies of G143A isolates present in field populations of *P. tritici-repentis*, requiring the use of a QoI fungicide mixed with a non-cross-resistant single-site fungicide such as a DMI fungicide or a fungicide with multi-site activity (inhibitors), such as chlorothalonil to effectively control the disease (Sierotzki et al., 2007).

It was discovered in 2007 that 40-50% of *P. tritici-repentis* isolates tested in Denmark were found to have EC_{50} values for strobilurin fungicides of over 10 ppm (Jørgensen, 2008). This level of insensitivity to strobilurins is associated with a high frequency of the G143A mutation within the field populations of *P. tritici-repentis*, and as such triazoles have been mostly recommended in Europe for control of tan spot over strobilurins, due to the latter's ability to control the disease (Jørgensen, 2008). Certain plant pathogens have not displayed insensitivity to QoI products despite strong selection pressure due to the presence of an intron that occurs after amino acid position 143 in the cytochrome *b* gene, which prevents substitution from taking place. One such group of diseases that has no insensitivity to QoI products due to this phenomenon are the rusts (Grasso et al., 2006).

Strobilurin fungicides inhibit mitochondrial electron transfer by binding to the QoI centre of the cytochrome bc_1 complex, interfering with ATP synthesis (Ulrich et al., 1988). Several plant pathogens are able to resist these fungicides by inducing an alternative oxidase pathway, allowing ATP synthesis to continue by circumventing the cytochrome *b* site of QoI action (Vanderberghe and Mcintosh, 1997). *Phaeosphaeria nodorum* isolates have been found that possess high EC_{50} values while lacking the G143A substitution (Blixt et al., 2009). This may be due to the fungus accessing an alternative oxidase pathway, as fungi making use of this pathway have been found to

better tolerate QoI fungicides in vitro (Wise et al., 2008; Ziogas et al., 1997). Although this process appears to function *in vitro,* it does not appear to decrease the efficacy of the fungicide in the field (Tamura et al., 1999). Olya et al. (1998) theorized that plant antioxidants present in the host plant impeded reactive oxygen species from the plant pathogen from reaching levels that would induce the alternative oxidase pathway during QoI action in the field. Salicylhydroxamic acid, otherwise known as SHAM acts as an inhibitor of the alternative oxidase pathway under laboratory conditions (Jin et al., 2009). It has been documented that SHAM has no effect on conidial germination (Wise et al., 2008), however Rebollar-Alviter et al. (2007) found that SHAM on average inhibited radial growth of *Phytophthora cactorum* by 30%. EC_{50} values of percent conidial germination have previously been found to be an effective method of determining QoI fungicide sensitivity in pathogen isolates (Pasche et al., 2004; Wong and Wilcox, 2000) and has been used to develop a fungicide sensitivity baseline for *A. rabiei* (Wise et al., 2008). These methods are often extremely labour intensive as a large number of samples must be tested to determine pathogen resistance level in a population (Russel, 2004).

A loss of sensitivity to QoI fungicides in fungicide-exposed isolates of 100-fold or greater compared to non-exposed baseline samples is often a sign the G143A mutation is present. The G143A mutation has been reported in a large number of different pathogens across Europe, Asia, and North America. (Avila-Adame et al., 2003 ; Brasseur et al., 1996 ; Brent and Holloman, 2007 ; Fraaije et al., 2002 ; Gisi et al., 2002 ; Ishii et al., 2001 ; Kianianmomeni et al., 2007 ; Kim et al., 2003 ; Wong et al., 2007). While resistance to DMI and carboximide fungicides is only considered a medium risk, pathogens have developed resistance to both of these fungicides (Avenot and Michailides, 2007; Luo and Schnabel, 2008). There have also been reports of cross-class resistance among isolates, as DMI-resistant samples of *Monilinia fructicola* became resistant to azoxystrobin,

a QoI fungicide, more rapidly than non DMI-sensitive isolates (Luo and Schnabel, 2008). Reimann and Deising (2004) also found that isolates of *P. tritici-repentis,* once adapted to either DMI or QoI fungicides became cross-resistant, and would grow on either agar amended with either fungicide or infect leaves treated with either fungicide.

2.5 *Pyrenophora tritici-repentis* Genetics

Pathogen populations evolve rapidly when confronted with changing environments when the population has large genetic variation and may display increased virulence against host plants or reduced sensitivity to fungicides (Patel et al., 2012). *Pyrenophora tritici-repentis* has been classified into 11 races based on virulence as well as wheat differential genotypes (Singh and Hughes 2006). Many *P. tritici-repentis* isolates have demonstrated mycotoxigenic properties on wheat, producing various anthraquinone mycotoxins which contaminate kernels in the field as opposed to in storage (Bouras et al., 2009). A thorough understanding of the *P. tritici-repentis* population structure is necessary to assess the probability that isolates with new virulence characteristics may emerge, as well as the probability that resistance to fungicides will develop.

The pathogenicity of *P. tritici-repentis* is closely associated with its ability to produce three toxins; ToxA, ToxB, and ToxC, which are each host-selective (Lamari and Strelkov 2010). All three of these toxins cause either chlorosis or necrosis when they interact with specific susceptible wheat genotypes. ToxA causes necrosis on wheat, while ToxB and ToxC cause chlorosis, albeit on different wheat cultivars (Lamari and Strelkov 2010). There are eight races of *P. tritici-repentis*, which produce these toxins at different levels and in different combinations. In Canada, as well as the United States and Australia, the races of *P. tritici-repentis* that are most prevalent are Races 1 and 2, that do not carry the gene required to produce ToxB, however all eight races exist in the Fertile Crescent (Lamari and Strelkov 2010). Ptr ToxA is produced by the vast majority of isolates

that have been collected in western Canada over the past several decades (Lamari and Bernier 1989; Lamari et al., 1998). Ptr Tox A is the most damaging of the Ptr toxins to wheat cultivars produced in Canada as the majority of them are susceptible (Lamari et al., 1998). Although Ptr ToxC is produced by nearly half of the *P. tritici-repentis* population in western Canada, the majority of wheat produced in the country is resistant to Race 3 and therefore insensitive to Ptr ToxC. Ptr ToxB is not believed to be an issue in western Canada as the races capable of producing the toxin, Races 5, 6, 7, and 8 are absent except for the low-virulence Race 5 (Lamari et al., 1998; Strelkov et al., 2002). However, races that produce ToxB have increased recently in the US and nearly all Canadian wheat varieties are susceptible to ToxB (Lamari and Strelkov 2010). Races 1 and 2 cause similar effects on both hexaploid and durum wheat, while Races 3 and 5 cause chlorosis in hexaploid wheat and necrosis in durum (Gamba and Lamari 1998). Races 3 and 5 are also more prevalent on durum compared to hexaploid wheat (Ali and Francl 1999). Ali and Francl (2003) suggested that native grasses may act as secondary hosts for the fungi to overwinter and may be an added source of genetic variation.

A study conducted by Di Zinno et al. (1998) determined that a limited number of *P. tritici-repentis* isolates contained high levels of polymorphisms, but could not detect a relationship between random amplifiable polymorphic DNA (RAPD) polymorphism and pathogenicity, geographic origin, or toxin production. Singh and Hughes (2006) reported that among 66 isolates collected across Saskatchewan, 97% of the isolates tested by DNA analysis using PCR were of Races 1 and 2. They also reported that isolates collected from fields close to each other had similarity coefficients ranging from 69% to 88%, while those collected from fields distant from each other ranged from 59% to 93% and that genetic variation was greater among isolates (96.8%) than races (3.2%). They noted that isolates collected from Western Canada possessed large genetic

variability, although most variability was among isolates regardless of virulence. They noted that the high level of genetic variability among *P. tritici-repentis* isolates in Western Canada is due in part to sexual recombination as the pathogen undergoes a sexual stage on wheat stubble between crops in addition to an asexual stage that occurs repeatedly during the growing season.

2.6 Testing for Insensitivity

Fungicide insensitivity in pathogen populations may arise due to both single and multiple gene mutations (Ma and Michailides, 2005). Fungicide insensitivity often occurs due to a low level of mutation in the pathogen population, however insensitive isolates may become dominant due to fungicides effectively controlling sensitive isolates, placing high selection pressure on the pathogen population over time (Ma and Michailides, 2005). Fungicide sensitivity may be conferred by several mechanisms (Gisi et al., 2000; Gullino et al., 2000; Fluit et al., 2001; McGrath, 2001) that include: (I) alteration of the target site, reducing binding of the fungicide; (II) synthesizing an alternative enzyme that substitutes for the target enzyme; (III) overproducing the fungicide target; (IV) reduced fungicide uptake or use of an active efflux; and (V) metabolic breakdown of the fungicide. The most common mechanism seen in fungicide insensitive phytopathogenic fungi is the alteration of a biochemical target site of the fungicide, such as the G143 and F129L mutations seen in many fungi populations (Gisi et al., 2002).

Molecular biology advances have resulted in several methods for rapidly detecting fungicide-insensitive isolates once the mechanism of insensitivity has been uncovered. These molecular techniques include PCR, PCR-restriction fragment length polymorphism (PCR-RFLP), and allele-specific real-time PCR. Quinone outside inhibitor (QoI) fungicide sensitivity has been monitored with the use of molecular techniques, as the G143A marker, which has successfully been used to monitor sensitivity in several pathogen populations (Bartlett et al., 2002; Gisi et al.,

2002; Russel, 2004). Ma and Michailides (2005) noted that using allele-specific real-time PCR allowed them to determine frequencies of azoxystrobin-insensitive alternaria in 60 orchards in the span of two days. A similar feat would require 200 workdays using conventional spore germination techniques and would require previous baseline sensitivity work to determine if insensitivity had occurred within the population.

CHAPTER 3

3. Determining Optimal Fungicide Timing to Control Leaf Spotting Diseases of Spring Wheat

3.1 Introduction

There are several leaf spotting diseases that affect spring wheat, including tan spot, caused by *Pyrenophora tritici-repentis* and pathogen species that constitute the septoria leaf spot complex. These include *Mycosphaerella graminicola* (anamorph: *Septoria tritici*), causal agent of septoria leaf blotch as well as *Phaeosphaeria nodorum* (anamorph: *Stagonospora nodorum*) and *Phaeosphaeria avenaria* (anamorph: *Stagonospora avenae*), causal agents of stagonospora blotch (Goodwin, 2012). Tan spot may lead to yield losses of 50% in wheat in severe cases (Hosford, 1971; Hosford and Busch, 1974; Rees et al., 1982; Rees and Platz, 1983; Tekauz and Platford, 1982); however, losses as high as 75% have been recorded (Rees et al., 1981). Yield losses of 30-50% have been attributed to septoria leaf blotch during severe epidemics, however, yield losses are usually significantly lower (Eyal et al., 1987). Stagonospora blotch infection leads to minor yield loss, most often resulting in 10-15% lower production overall (King et al., 1983).

Environmental and cultural practices play a large role in the severity of leaf spotting diseases. For instance, Evans et al. (1999) reported that leaf spots reduced yields of inoculated plots by 15% in Oklahoma, which was similar to findings made by Shabeer and Bockus (1988) in Kansas, who saw yield loses of 17%. Rees et al. (1982), however, reported yield losses of nearly 50% in control plots planted in Australia.

Fusarium head blight (*Fusarium graminearum* Schwabe, group II [anamorph]) (FHB) is a major disease that has been gaining prominence over the previous two decades across the Canadian Prairies and the Pacific northwest of the United States. Fusarium head blight results in the entire

ear or a portion of the ear to undergo premature senescence, causing a reduction in yield and grain quality (Haidukowski et al., 2005). Yield losses of over 70% have been reported when FHB reached epidemic levels. *Fusarium graminearum* is capable of producing a wide assortment of mycotoxins, the most common being deoxynivalenol (DON) (Bottalico and Perrone, 2002). DON is neurotoxic and immunotoxic and even low levels can have detrimental effects on both humans and livestock (FAO/WHO, 2001). The European Union has placed limits on the acceptable DON levels in wheat and cereal products packaged for human consumption (Koornneef et al., 2002).

Fungicide application is strategically applied to protect the flag leaf from pathogen infection (Wegulo et al., 2012). Most fungicides are applied to prevent pathogen infection, and are useless once disease symptoms have appeared (McGrath, 2004). Fungicide application during a time that ensures the protection of the flag leaf is paramount, as the flag leaf is responsible for the bulk of the grain filling in wheat (Ruske et al., 2003; Simpson, 1968). The optimal time to apply fungicide in wheat has been highly contested, although several studies have suggested that early fungicide application is imperative for improving yield (Wegulo et al., 2012). Optimal Fungicide timing to control FHB has been suggested to be at anthesis stage (ZGS60) (Halley et al., 2001). The optimal timing to control leaf spots, however, has been reported to be at flag leaf stage (ZGS39) (Wiersma and Motteberg, 2005).

Prothioconazole + tebuconazole has been recommended to control FHB as it has been shown to be more effective than tebuconazole alone at reducing DON levels (Paul et al., 2007). Fungicide application studies conducted by Martin and Johnson (1982), determined that FHB was reduced by the addition of propiconazole, resulting in a 34% increase in yield when applied during anthesis. Similar tests conducted by Boyacioglu et al. (1992) found that FHB severity was reduced by 39-61% through application of triadimefon and propiconazole at the flowering stage.

Additionally, experiments conducted in both Minnesota and North Dakota discovered that tebuconazole was the most effective of the triazole fungicides, reducing fusarium head blight severity by 71% when applied at heading stage (McMullen et al., 1997a; McMullen 1998).

The objective of this study was to determine whether applying fungicide at anthesis stage (ZGS60) would adequately control leaf spots compared to applying fungicide at flag leaf stage (ZGS 39). Determining whether applying fungicide at anthesis stage, the recommended timing to control FHB, would also adequately control leaf spots would be beneficial to growers. It would allow them to apply fungicide at only one timing rather than two to control leaf spotting diseases of spring wheat, thereby gaining an economic benefit while at the same time reducing the risk of development of fungicide insensitive pathogens due to overexposure.

3.2 Materials and Methods

The study was conducted at Saskatoon, Indian Head and Melfort in Saskatchewan, as well as Lethbridge and Brooks in Alberta in 2013. The study was repeated in 2014 at Saskatoon and Melfort in Saskatchewan and at Lethbridge, Brooks and Lacombe in Alberta. Plots were established at Saskatoon in a field previously sown to canola in 2012 and wheat in 2013, at Indian Head in a field previously sown to wheat in 2012, at Melfort in a field previously sown to canola in 2012 and 2013, at Brooks in a field previously sown to alfalfa in 2012 and summer fallow in 2013, at Lethbridge in a field previously sown to barley in 2012 and 2013 and at Lacombe in a field previously sown to wheat in 2013. Prior to seeding, glyphosate (900 g a.e. ha^{-1}) was applied to the entire plot area to suppress weeds at all sites. Subsequent herbicide applications were applied with various herbicides at various timings to control weeds as necessary at each site. Soil samples were collected at each site and fertilizer applied to achieve 100% of the soil test recommendations.

The plot sizes were 2 x 8 m at Saskatoon (52.1332°N, 106.6700°W, black soil zone), 1.4 x 6 m at Indian Head (50.5334°N, 103.6699°W, black soil zone), 4 x 10 m at Melfort (52.8608°N, 104.6143°W, black soil zone), 1.2 x 6 m at Brooks (50.5334°N, 111.8992°W, brown soil zone), 2 x 6 m at Lethbridge (49.6935°N, 112.8418°W, brown soil zone) and 1 x 5.5 m at Lacombe (52.4631°N, 113.7286°W, brown soil zone). Seeding depth at each site varied from 3.81 cm to 7.62 cm, while row spacing varied from 20.3 to 24.1 cm and seeding rate from 250 to 275 seeds m^2. Experiments were designed as randomized complete blocks of four replicates. Each trial consisted of a 3 x 5 factorial plus an unsprayed check, for a total of 16 treatments. The fungicide treatments were: 1) prothioconazole + tebuconazole (Prosaro® at 800 mL ha^{-1}), 2) tebuconazole (Folicur® 250 EW at 499 mL ha^{-1}, or Folicur® 432 F at 291 mL ha^{-1} [Lacombe 2014]), 3) prothioconazole + tebuconazole (400 mL ha^{-1}) tank mixed with *Bacillus subtilis*, a biological fungicide (Serenade Optimum®, Bayer CropScience at 500 g ha^{-1}), and 4) tebuconazole (375 mL ha^{-1}) tank mixed with *B. subtilis* (500 g ha^{-1}) and 5) *B. subtilis* alone (500 g ha^{-1}), all applied in with 100 L ha^{-1} of water. Three fungicide application timing treatments consisted of a single application at flag leaf stage (ZGS39), a single application at anthesis (ZGS60) and an application at each ZGS39 and ZGS60.

The CWRS wheat variety Carberry was chosen as it is moderately susceptible to leaf spotting diseases, but moderately resistant to FHB (Anon, 2014). This choice of variety was made to help reduce the confounding effects between leaf spots and FHB. Disease ratings were conducted at each application date and prior to the fungicide treatment. Leaf spotting disease severity was determined by collecting 10 flag leaf and 10 penultimate leaves from each plot and rated using the Horsfall – Barratt scale (0-11) (Horsfall and Barratt 1945). Ratings were then converted to percent leaf area affected by the disease. Fifty random spikes were collected from

each plot and the Stack and McMullen scale (Stack and McMullen, 1995) was used to determine disease severity of FHB by the following calculation: disease severity = $\sum i\ (n_i x_i)/N$, where n_i is the number of plants in class i, and N is the total number of plants assessed. Fusarium head blight incidence was expressed as the percentage of heads with symptoms of FHB.

Wheat was harvested from a 10-42 m^2 area from the center row of each plot using a small-plot combine, with the outside rows of each plot excluded. Samples were air-dried for approximately 48 hours, adjusted to a moisture content of 14.5% and cleaned. Harvest data included plot yield (kg ha^{-1}), test weight (kg hl^{-1}), and thousand kernel weight (g). A subsample from the yield sample of each plot was analyzed to determine protein content (12.5% moisture content) using LECO protein analyser, which uses the Dumas method that combusts a sample of known mass at 900^{o}C in a chamber in the presence of oxygen. Carbon dioxide, water, and nitrogen are released and the gases are passed over columns containing a potassium hydroxide aqueous solution that absorbs the carbon dioxide and water. A thermal conductivity detector is then used to separate the nitrogen from residual carbon dioxide and water and the nitrogen is measured.

All statistical analyses were performed using the mixed procedure of SAS version 9.3 statistical software (SAS Institute Inc., Cary, NC), and treatment means separated with the Least Significance Difference (LSD) test ($P < 0.05$). The effects of treatments were considered fixed effects, and location and year, as well as blocks within location*year were considered random effects. The DDFM = kenwardroger option was considered for approximating the degrees of freedom for means. Analyses of variances are presented in Appendices XLVI to LXIII Contrast statements were used to make comparisons among treatments of interest.

Precipitation values were collected for each site-year for the growing period from April until August (Table 3.1). The high disease locations had 3 of the 4 highest overall precipitation

values during the growing season and had an average rainfall amount of 293.2 mm compared to the low disease locations, which had an average rainfall amount of 213.6 mm.

Table 3.1: Precipitation values for growing season at each site location.

Location	April (mm)	May (mm)	June (mm)	July (mm)	August (mm)	Total (mm)
Saskatoon 2013	10.5	15.9	117.7	35.6	14.9	194.6
Saskatoon 2014	74.2	61.1	94.8	44.5	18.5	293.1
Melfort 2013	5.8	19.0	97.9	103.2	11.7	237.6
Melfort 2014	50.3	24.3	167.3	38.8	57.9	338.6
Indian Head 2013	7.6	18.0	105.0	50.9	7.1	188.6
Brooks 2013	23.1	38.9	92.2	53.7	17.3	225.2
Brooks 2014	21.9	29.6	93.2	33.7	29.8	208.2
Lethbridge 2013	9.5	81.9	82.0	49.9	35.0	258.3
Lethbridge 2014	29.6	38.1	49.5	16.7	49.0	182.9
Lacombe 2014	22.4	45.0	83.2	72.6	24.7	247.9

3.3 Results

The data collected from each site-year were classified into high disease locations, where leaf spot disease severity of the unsprayed check was 40% or greater, and low disease locations, where leaf spot disease severity of the unsprayed check was less than 40%. Data from high disease severity site-years was combined, as was data from low disease severity site-years, and each analyzed separately. High disease locations included: Saskatoon, Melfort, and Lacombe, all in 2014, while all other site-years were considered low disease severity locations. Melfort 2013 was analyzed separately as the unusually high FHB severity was confounded with leaf spot severity.

3.3.1 High leaf spot disease severity site-years

The mean leaf spot disease severity of the unsprayed check in the high disease locations was 73.2%, which was dramatically reduced to 31.8% on average with the application of fungicide (Table 3.2). There was a significant difference in leaf spot disease severity between fungicide

treatment at the flag leaf stage (34.7%) and treatment at both timings (20.2%), as well as between the anthesis timing treatment (41.0%) and the treatment at both timings and between fungicide treatment at the flag leaf stage compared to anthesis timing (Tables 3.2 and 3.3).

Table 3.2: Mean values of contrast groups for harvest data at high disease severity locations: Melfort, Saskatoon and Lacombe in 2014.

Treatment	Final Leaf Disease (%)	FHB (%)	Yield (kg ha^{-1})	Test Weight (kg hL^{-1})	Thousand Kernel Weight (g)	Protein (%)
Fungicide Timing Treatments						
Control	73.2 ± 4.1	3.2 ± 1.0	3676 ± 233.6	78.2 ± 0.6	33.4 ± 0.4	14.8 ± 0.2
Flag leaf	34.7 ± 2.5	3.6 ± 0.6	4010 ± 163.0	78.3 ± 0.3	35.4 ± 0.3	15.3 ± 0.3
Anthesis	41.0 ± 3.3	4.0 ± 0.6	4141 ± 131.6	78.6 ± 0.3	36.0 ± 0.3	15.2 ± 0.2
Both timings	20.2 ± 2.3	3.1 ± 0.6	4246 ± 156.1	78.6 ± 0.3	36.6 ± 0.3	15.5 ± 0.2
Fungicide Product Treatments						
B. subtilis	68.9 ± 3.2	3.4 ± 0.6	3346 ± 141.0	78.0 ± 0.3	33.3 ± 0.3	15.0 ± 0.2
Full rate	28.1 ± 2.3	3.2 ± 0.5	4192 ± 123.2	78.5 ± 0.2	36.0 ± 0.2	15.3 ± 0.2
Reduced rate tank mix	35.5 ± 2.5	3.6 ± 0.5	4074 ± 123.5	78.5 ± 0.3	36.0 ± 0.2	15.3 ± 0.2
Fungicides combined	31.8 ± 1.7	3.6 ± 0.3	4133 ± 87.0	78.5 ± 0.2	36.0 ± 0.2	15.3 ± 0.1
Prothioconazole + tebuconazole	27.6 ± 2.4	3.9 ± 0.5	4136 ± 130.1	78.5 ± 0.3	36.3 ± 0.2	15.3 ± 0.2
Tebuconazole	36.1 ± 2.4	3.2 ± 0.4	4130 ± 116.5	78.5 ± 0.2	35.7 ± 0.2	15.4 ± 0.2

Full rate included either a full rate of prothioconazole + tebuconazole or tebuconazole. Reduced rate tank mix included reduced rates of either prothioconazole + tebuconazole or tebuconazole mixed with 500 g of *B. subtilis.* Fungicides combined included full rates of prothioconazole + tebuconazole or tebuconazole as well as reduced rates of prothioconazole + tebuconazole and tebuconazole with 500 g *B. subtilis.* Prothioconazole + tebuconazole included a full rate of prothioconazole + tebuconazole as well as reduced rate of prothioconazole + tebuconazole with 500 g *B. subtilis.* Tebuconazole included a full rate of tebuconazole as well as a reduced rate tebuconazole with 500 g *B. subtilis.*

There were also effects of fungicides on leaf spot disease severity, tebuconazole reduced severity to 36.1%, while prothioconazole with tebuconazole reduced disease severity to 27.6% (Tables 3.2 and 3.3). Applying a full-rate of fungicide was also more effective to control leaf spot

disease severity compared to a reduced rate mixed with the bio-fungicide *B. subtilis* (Tables 3.2 and 3.3). The full-rate reduced disease severity to 28.1%, while the reduced rate was slightly less effective, reducing disease severity to 35.5% from 73.3% on the unsprayed check. No differences were found in FHB incidence among any of the fungicide treatments.

Table 3.3: *P*-values for fungicide treatment comparisons using simple linear contrasts on leaf spots of wheat for high disease, including: Melfort, Saskatoon and Lacombe in 2014.

Contrast	Leaf disease (%)	FHB (%)	Yield (kg ha^{-1})	Test weight (kg hL^{-1})	Thousand kernel weight (g)	Protein (%)
Flag vs. anthesis	**0.0198***	0.6315	0.2343	**0.0008*****	**0.0156***	0.6627
Flag vs. both	**<0.0001****	0.3829	**0.0267***	**0.0001*****	**<0.0001****	0.3994
Anthesis vs. both	**<0.0001****	0.1771	0.2987	0.5788	**0.0277***	0.2037
Unsprayed vs. biological	0.2861	0.9270	**0.0022****	0.2135	0.8835	0.5482
Full-rate vs. reduced rate	**0.0003*****	0.7532	0.1812	0.7624	0.7307	0.9113
Unsprayed vs. fungicide	**<0.0001****	0.7139	**0.0044****	**0.0139***	**<0.0001****	0.1201
Prothioconazole + tebuconazole vs. tebuconazole	**<0.0001****	0.1201	0.9470	0.7338	**0.0033****	0.7384

There was a difference in yield between the unsprayed check and the fungicide treatments. The unsprayed check had a mean yield of 3676 kg ha^{-1} and the fungicide treatments 4133 kg ha^{-1} (Tables 3.2 and 3.3). There was also a significant difference between the flag leaf treatment and the treatment at both timings, with yield of the flag leaf treatment of 4010 kg ha^{-1}, whereas the treatment at both timings had an average yield of 4246 kg ha^{-1} (Tables 3.2 and 3.3).

There was a slight, but significant difference in test weight (kg hL^{-1}) between the unsprayed check (78.2 kg hL^{-1}) and the fungicide treatments (78.5 kg hL^{-1}) (Tables 3.2 and 3.3). There was also a difference between the flag leaf treatment (78.3 kg hL^{-1}) compared to the anthesis treatment (78.6 kg hL^{-1}) and the treatment at both timings (78.6 kg hL^{-1}). There was a difference in thousand

kernel weight (TKW) between the unsprayed check (33.4 g) and fungicide treatments (36.0 g) (Table 3.2). There was also a slight difference in TKW between plots sprayed at the flag leaf stage (35.4 g) and plots sprayed during anthesis (36.0 g) and both timings (36.6 g) (Tables 3.2 and 3.3) and between the prothioconazole with tebuconazole treatment (36.3 g) and the tebuconazole treatment (35.8 g) (Tables 3.2 and 3.3). There were no differences in protein content among any of the fungicide treatments.

3.3.2 Low leaf spot disease severity site-years

The mean leaf spot disease severity of the unsprayed check in the low disease locations was 32.3%, which was reduced to 11.8% on average with the application of fungicide (Table 3.4). Plots sprayed with the biological fungicide or chemical fungicide had lower FHB disease severity compared to the unsprayed check. The average FHB disease severity of the unsprayed check was 0.6% versus 0.1% in the plots treated with the biological fungicide and 0.1% in the plots treated with chemical fungicide, respectively. However, these values are too small to be considered biologically significant. FHB levels were very low in all treatments and appeared to have no influence on any other factors measured. Plots treated at both timings had a slightly higher TKW, at 40.9 g compared to plots treated at flag leaf stage, with an average TKW of 40.3 g. The fungicide treatments had no effect on yield, TW or protein content (Table 3.5).

Table 3.4: Mean values of contrast groups for harvest data at low disease locations, including: Saskatoon, Indian Head and Brooks in 2013 and Lethbridge in 2013 and 2014.

Treatment	Yield (kg ha^{-1})	Final Leaf Disease (%)	FHB (%)	Test Weight (kg hL^{-1})	Thousand Kernel Weight (g)	Protein (%)
Fungicide Timing Treatments						
Control	4534 ± 296.2	32.3 ± 3.5	0.60 ± 0.60	75.5 ± 2.6	40.5 ± 0.7	14.3 ± 0.4
Flag leaf	4519 ± 128.2	12.5 ± 0.7	0.06 ± 0.07	75.0 ± 1.2	40.3 ± 0.3	14.3 ± 0.2
Anthesis	4528 ± 126.1	12.6 ± 0.8	0.01 ± 0.05	75.8 ± 1.1	40.6 ± 0.3	14.2 ± 0.3
Both timings	4518 ± 127.3	10.3 ± 0.7	0.05 ± 0.04	75.1 ± 1.2	40.9 ± 0.3	14.5 ± 0.2
Fungicide Product Treatments						
Biological	4490 ± 161.3	27.9 ± 1.3	0.05 ± 0.06	75.1 ± 1.5	40.2 ± 0.4	14.3 ± 0.3
Full rate	4549 ± 117.8	12.0 ± 0.5	0.10 ± 0.07	75.4 ± 1.0	40.6 ± 0.3	14.2 ± 0.2
Reduced rate tank mix	4496 ± 115.3	11.6 ± 0.5	0.02 ± 0.02	75.1 ± 1.1	40.6 ± 0.3	14.5 ± 0.2
Fungicide	4523 ± 82.3	11.8 ± 0.3	0.07 ± 0.04	75.3 ± 0.7	40.6 ± 0.2	14.4 ± 0.2
Prothioconazole + tebuconazole	4530 ± 117.2	10.8 ± 0.5	0.06 ± 0.03	75.4 ± 1.0	40.7 ± 0.3	14.4 ± 0.2
Tebuconazole	4515 ± 116.0	12.8 ± 0.5	0.09 ± 0.07	75.1 ± 1.1	40.5 ± 0.3	14.3 ± 0.2

Full rate included either a full rate of prothioconazole + tebuconazole or tebuconazole. Reduced rate tank mix included reduced rates of either prothioconazole + tebuconazole or tebuconazole mixed with 500 g of *B. subtilis.* Fungicides combined included full rates of prothioconazole + tebuconazole or tebuconazole as well as reduced rates of prothioconazole + tebuconazole and tebuconazole with 500 g *B. subtilis.* Prothioconazole + tebuconazole included a full rate of prothioconazole + tebuconazole as well as reduced rate of prothioconazole + tebuconazole with 500 g *B. subtilis.* Tebuconazole included a full rate of tebuconazole as well as a reduced rate tebuconazole with 500 g *B. subtilis.*

Table 3.5: *P*-values for fungicide treatment comparisons using simple linear contrasts on leaf spots of wheat for low disease, including: Saskatoon, Indian Head and Brooks in 2013 and Lethbridge in 2013 and 2014.

Contrast	Yield (kg ha^{-1})	Leaf Disease (%)	FHB (%)	Test Weight (kg hL^{-1})	Thousand Kernel Weight (g)	Protein (%)
Flag vs. anthesis	0.8868	0.9910	0.7787	0.1240	0.2668	0.6856
Flag vs. both	0.9488	0.2145	0.8735	0.7868	**0.0202***	0.5355
Anthesis vs. both	0.8362	0.2104	0.6597	0.2044	0.2214	0.3064
Unsprayed vs. biological	0.5762	0.1316	**0.0050****	0.6601	0.5264	0.8768
Full-rate vs. reduced rate	0.1755	0.7615	0.2812	0.5374	0.9557	0.1777
Unsprayed vs. fungicide	0.8733	**<.0001*****	**0.0032****	0.7999	0.7763	0.9886
Prothioconazole + tebuconazole vs. tebuconazole	0.6942	0.1837	0.7422	0.4556	0.4810	0.7176

3.3.3 Melfort, 2013

The FHB severity at Melfort 2013 was high (38.5%) and for this reason this site was analysed separately from the other low leaf spot disease severity locations (Table 3.6). The high FHB level seen in Melfort may be due to the fact that the anthesis spraying was 5 days late due to anthesis beginning on the weekend and rain occurring early in the week. Despite the much higher FHB disease severity and similar leaf spot disease severity, Melfort 2013 averaged 938 kg ha^{-1} higher yield and 8.4 kg hL^{-1} higher TW than the unsprayed check compared to the other low disease locations (Table 3.6). There was no difference in FHB severity between treatments and it can be assumed that FHB had a similar effect on all treatments for all factors measured (Table 3.7).

Leaf spot disease severity at Melfort in 2013 was 33.3% in the unsprayed check, which was reduced to 10.5% with the application of fungicide (Table 3.6). There was a difference in leaf spot disease severity when fungicide was applied at the flag leaf stage (12.0%) versus at both timings (5.8%) and when fungicide was applied at anthesis (13.7%) versus both timings (Tables

3.6 and 3.7). There was also a slight reduction in both TW and TKW when fungicide was applied at flag leaf stage (83.8 kg hL^{-1} and 38.3 g, respectively) compared to fungicide application at anthesis (84.0 kg hL^{-1} and 39.5 g, respectively) and TW when fungicide was applied at anthesis (84.0 kg hL^{-1}) compared to both timings (83.8 kg hL^{-1}) (Table 3.6 and 3.7).

Table 3.6: Mean values of contrast groups for harvest data at Melfort in 2013.

Treatment	Yield (kg ha^{-1})	Final Leaf Disease (%)	FHB (%)	Test Weight (kg hL^{-1})	Thousand Kernel Weight (g)	Protein (%)
Fungicide Timing Treatments						
Control	5472 ± 192.1	33.3 ± 3.7	38.5 ± 3.1	83.9 ± 0.09	38.8 ± 0.5	13.7 ± 0.2
Flag leaf	5417 ± 54.4	12.0 ± 1.2	40.9 ± 1.8	83.8 ± 0.05	38.3 ± 0.3	14.2 ± 0.1
Anthesis	5486 ± 42.3	13.7 ± 1.7	36.8 ± 1.9	84.0 ± 0.05	39.5 ± 0.3	14.1 ± 0.1
Both timings	5452 ± 58.3	5.8 ± 0.5	40.0 ± 2.1	83.8 ± 0.05	38.6 ± 0.3	14.0 ± 0.1
Fungicide Product Treatments						
Biological	5394 ± 76.2	36.7 ± 3.1	42.7 ± 2.3	83.8 ± 0.09	38.2 ± 0.4	14.0 ± 0.1
Full rate	5470 ± 42.1	10.5 ± 1.4	37.4 ± 1.4	83.9 ± 0.03	38.8 ± 0.3	14.2 ± 0.08
Reduced rate tank mix	5433 ± 42.5	10.5 ± 1.1	41.0 ± 1.8	83.8 ± 0.06	38.8 ± 0.3	14.0 ± 0.09
Fungicide	5452 ± 29.7	10.5 ± 0.9	39.2 ± 1.1	83.8 ± 0.03	38.8 ± 0.2	14.1 ± 0.06
Prothioconazole + tebuconazole	5449 ± 39.8	10.1 ± 1.3	40.1 ± 1.7	83.8 ± 0.06	38.8 ± 0.3	14.1 ± 0.08
Tebuconazole	5454 ± 45.0	10.9 ± 1.2	38.4 ± 1.6	83.8 ± 0.04	38.8 ± 0.3	14.1 ± 0.09

Full rate included either a full rate of prothioconazole + tebuconazole or tebuconazole. Reduced rate tank mix included reduced rates of either prothioconazole + tebuconazole or tebuconazole mixed with 500 g of *B. subtilis.* Fungicides combined included full rates of prothioconazole + tebuconazole or tebuconazole as well as reduced rates of prothioconazole + tebuconazole and tebuconazole with 500 g *B. subtilis.* Prothioconazole + tebuconazole included a full rate of prothioconazole + tebuconazole as well as reduced rate of prothioconazole + tebuconazole with 500 g *B. subtilis.* Tebuconazole included a full rate of tebuconazole as well as a reduced rate tebuconazole with 500 g *B. subtilis.*

Table 3.7: *P*-values for fungicide treatment comparisons using simple linear contrasts on leaf spots of wheat for Melfort in 2013.

Contrast	Yield (kg ha^{-1})	Leaf disease (%)	FHB (%)	Test weight (kg hL^{-1})	Thousand kernel weight (g)	Protein (%)
Flag vs. anthesis	0.2250	0.4765	0.1370	**0.0095****	**0.0076****	0.5385
Flag vs. both	0.5342	**0.0086****	0.7608	0.8065	0.4387	0.3954
Anthesis vs. both	0.5490	**0.0012****	0.2337	**0.0176***	0.0501	0.8127
Unsprayed vs. biological	0.3904	0.3639	0.3485	0.4252	0.4085	0.1567
Full-rate vs. reduced rate	0.4180	0.9627	0.1116	0.3198	0.9805	0.1924
Unsprayed vs. fungicide	0.8039	**<.0001*****	0.8574	0.4070	0.9946	0.0638
Prothioconazole + tebuconazole vs. tebuconazole	0.9233	0.6455	0.4347	0.9468	0.9416	0.6989

3.4 Discussion

The most effective treatment for increasing yield was fungicide application at both timings, which increased yield from 3676 kg ha^{-1} to 4246 kg ha^{-1}, a gain of 13.4% when disease levels were high. These yield increases were much lower than that reported by Wiersma and Motteberg (2005), who noted a 42% increase from 2854 kg ha^{-1} to 4910 kg ha^{-1}, when trifloxystrobin and propiconazole was applied at ZGS 31 (first node visible) and ZGS 39 (flag leaf stage). Ransom and McMullen (2008) also found that strobilurin fungicides improved yields between 5.5 and 44% in North Dakota. Lopez et al. (2015) noted an increase in yield between 800 kg ha^{-1} and 1690 kg ha^{-1} based on the fungicide treatments applied and suggested that two- and three-spray strategies provided the greatest yield increases. Fungicide increased yield and reduced leaf disease to a greater degree in these studies compared to what was observed in this study. For instance, Ransom and McMullen (2008) observed that fungicide application reduced leaf spot disease incidence from 81.5 to 10.9%, whereas the most effective treatment in our study, applying fungicide at both timings, reduced leaf disease severity from 73.2 % in the unsprayed check to 20.2% in the high

disease locations, 32.3% to 10.3% in the low disease locations and 33.3% to 5.8% at Melfort in 2013. The reason for this observed discrepancy in fungicide efficacy may be due to the fact that prior studies used both strobilurin and triazole fungicides, whereas our study used fungicides from the triazole family exclusively. Another possibility is that environmental conditions played a role as temperature, humidity and wind would have varied among locations. Fungicide efficacy varied among locations where our study was conducted, which may be due to the different environmental conditions experienced at each location, which included differences in total precipitation as well as distribution of rainfall across the growing season at each location.

Differences in leaf disease severity were observed when fungicide was applied at flag leaf timing compared to both timings, and when fungicide was applied at anthesis compared to both timings at both high disease and low disease locations. Differences in leaf disease severity between flag leaf and anthesis stage fungicide application were only observed in the high disease locations, however, applying fungicide at flag leaf stage only reduced leaf disease by 6% compared to fungicide application at anthesis and had no effect on yield. Cromey et al. (2004) observed no difference in control of ascochyta leaf spot (*Didymella exitialis*, anamorph: *Ascochyta* spp.) on barley when azoxystrobin and tebuconazole were applied at three separate timings between flag leaf stage and anthesis. Bockus et al. (1997) found that the optimum disease control occurred between the boot and fully headed growth stages. Duczek and Jones-Flory (1994), however, claimed the optimum time to apply fungicide was between the flag leaf and the medium milk growth stages. Wiersma and Motteberg (2005) found similar results to this study, noting that applying fungicide at ZGS 39 and ZGS 60 (anthesis) gave similar leaf spot disease control in two years between 2001 and 2003, while in one of the three years, applying fungicide at ZGS 60 actually provided greater control of disease than applying at ZGS 39.

Lopez et al. (2015) suggested that applying fungicide at ZGS 31 and again at ZGS 39 provided a greater yield increase than a single application at either ZGS 31 or ZGS 39 alone. Our results are similar, as applying fungicide at ZGS 39 and again at ZGS 60 increased yield and reduced disease severity compared to fungicide application at either timing individually. However, the increase in yield was minor and may not justify a second application when fungicide and application costs and the price of wheat are taken into account. Similar findings were reported by Wegulo et al. (2009) who determined that tan spot disease symptoms assessed during flowering had the strongest relationship with yield, compared to disease assessments conducted at seven other growth stages. Similar observations were made by both Shaner and Buchley (1995) from data collected in Indiana over a 19-year period, and by Milus and Chalkley (1997) who conducted work on plots infected with *P. nodorum*. These studies indicate that applying fungicide at or before flag leaf stage is unnecessary.

Application of fungicide at ZGS 38 (when the flag leaf is first visible) reduced the risk of leaf blotch (LB) reaching the flag leaf by 55-75%, whereas application at ZGS 39, the flag leaf stage, reduced risk by only 62-69% (Willyerd et al., 2015). In that study, a split application of a half-rate of fungicide at ZGS 13 (tillering) and at ZGS 38 reduced risk of LB reaching the flag leaf by 67-70%. They also noted that applying fungicide at ZGS 13 alone only reduced the risk of LB reaching the flag leaf between 32-37%. However, they did report that the ZGS 13 + ZGS 38 split-rate application provided a higher yield response than a single application at either ZGS 38 or ZGS 39. This would suggest, counter to our results and the results of several earlier studies, that an earlier split application is more effective at controlling disease. Our study has no treatment to compare to this study, however it was determined that applying fungicides at both ZGS 39 and ZGS 60 provided greater control and higher yields than applying fungicide at a single application

timing. The results of my study also demonstrated that there was no significant difference between a full-rate application and a reduced rate application on disease severity or yield, so it may be possible that a split application at a reduced rate is more effective for controlling leaf disease than a single application of fungicide. Applying the same fungicide family twice per growing season may also lead to fungicide insensitive pathogen. It was noted by Chang et al. (2007) that applying pyraclostrobin several times each year led to the selection of insensitive populations, while also contributing to the reduction in efficacy of pyraclostrobin to control ascochyta blight. As for the timing of application, applying fungicide during a wider range of growth stages should be used to determine the overall most effective timing to apply fungicide to improve yield and disease control. Lopez et al. (2015) stated that instead of trying to determine the ideal fungicide application timing, timing should be considered in relation to disease development. However, Paveley et al. (1997) has suggested that disease severity is an inconsistent measure for determining yield loss, even when multiple ratings are taken at various growth stages.

Differences were observed in leaf disease severity when applying prothioconazole with tebuconazole compared to tebuconazole when leaf disease severity was high. Prothioconazole with tebuconazole was 23.5% more effective at reducing disease severity than tebuconazole alone, however, no differences in yield were observed. Jørgensen and Thygesen (2006) suggested that based on disease control levels observed at ZGS 73 (early milk) and ZGS 77 (late milk), strobilurins such as pyraclostrobin, picoxystrobin and azoxystrobin provided greater control of tan spot than triazoles. Willyerd et al. (2015) however, reported no yield difference from plots treated with pyraclostrobin or prothioconazole with tebuconazole. Lopez et al. (2015) found that propiconazole and prothioconazole were the most effective triazoles. This is consistent with the

results of our study as prothioconazole with tebuconazole provided slightly better disease control than tebuconazole.

A difference in leaf disease severity was also observed between a full-rate and a reduced rate of fungicide mixed with 500 g of the bio-fungicide, *B. subtilis*, but no differences in yield were observed. The bio-fungicide alone proved to have no effect on disease severity, yield, TW, TKW or protein content compared to the unsprayed check. The full-rate of fungicide was 21% more effective in reducing leaf disease severity in the high disease locations but only increased yield by 118 kg ha^{-1}. Lopez et al. (2015) found that reduced rates of fungicide by between 25% and 50% of the recommended rate provided optimal yield returns. Jørgensen and Thygesen (2006) however, noted a significant decrease in disease control, from 90% to 75%, when fungicide was reduced from full-rates to quarter-rates.

Fusarium head blight severity was not reduced in either the high leaf spot disease severity site-years or at Melfort in 2013, however, both the biological fungicide and the chemical fungicide significantly reduced FHB severity at the low disease locations from 0.6% in the control to 0.1% in the biological fungicide treated plots and 0.1% in the chemical fungicide treated plots, respectively. However, the FHB severity in the low disease areas was very low and the reduction in FHB severity was marginal. Lopez et al. (2015) found similar results, noting that FHB severity was only marginally reduced with application of epoxiconazole with pyraclostrobin. This may be due to the fact that the fungicides used in these studies were specifically for the control of leaf spot diseases and were not registered for control of FHB.

Significant differences were observed for TKW when fungicide was applied at flag leaf stage, anthesis stage, or both timings at high leaf spot disease severity locations, between flag leaf stage and both timings at low disease locations, and between flag leaf and anthesis stages at Melfort

in 2013. Fungicide application at anthesis stage resulted in higher TKW than application at flag leaf stage and application at both timings resulted in the highest TKW compared to either single treatment of fungicide. It may be possible that applying fungicide only at flag leaf stage, as opposed to anthesis, resulted in more fusarium damaged kernels (FDK), as the optimal timing to control fusarium head blight is anthesis stage, resulting in lower TKW. When leaf spot severity was high, TKW was improved by fungicide treatment compared with the unsprayed check. Treatments sprayed with prothioconazole with tebuconazole, as opposed to tebuconazole also had higher TKW when leaf disease severity was high. Similar findings were reported by Wiersma and Motteberg (2005) who noted that fungicide application had a beneficial effect on kernel weight and test weight. Higher leaf disease would have resulted in less photosynthetic area, reducing grain filling and therefore reducing TKW.

Differences were observed in TW when leaf disease severity was high with the anthesis stage treatment having higher TW than the flag leaf stage treatment as well as the treatment at both timings having higher TW than the flag leaf stage treatment. The fungicide treatment also had higher TW compared to the unsprayed check, which may have been due to the benefit of applying fungicide, such as greater moisture content. Test weight was also slightly higher when fungicide was applied at anthesis compared to flag leaf stage or at both timings at Melfort in 2013.

There were no differences among fungicide treatments or between any of the fungicide treatments and the unsprayed check with respect to protein content in either the high disease or low disease locations. However, protein content was slightly higher in both the low disease locations and high disease locations when fungicide was applied compared to the unsprayed check. Ruske et al. (2003) made similar observations and suggested that fungicide application does not

lead to dilution of protein, which had been reported in previous studies. Wiersma and Motteberg (2005) also noted a slight increase in protein content when fungicide was applied.

3.5 Conclusion

Based on the findings of this study, it would appear that there was no difference in yield when applying fungicide at flag leaf stage compared to anthesis under either high or low leaf spot disease conditions. Disease severity was slightly lower when fungicide was applied at flag leaf stage compared to anthesis, however both TW and TKW were slightly higher when fungicide was applied at anthesis compared to flag leaf stage at the high disease locations, as well as at Melfort in 2013.

Applying fungicide at both timings resulted in marginally higher yield and significantly higher TW, TKW and lower disease severity compared to applying fungicide at flag leaf stage in the high disease locations and higher TKW and lower leaf spot disease severity in the low disease locations. Applying at both timings compared to anthesis also resulted in slightly higher yield and TKW and in the high disease locations and lower disease severity in both the high and low disease locations.

Since there was no yield difference between the reduced rate and full-rate fungicide applications it may be beneficial to apply a reduced rate of fungicide at both the flag leaf stage and anthesis stage when disease levels are high enough to warrant it. Further studies should be conducted to determine the benefits of applying a reduced rate of fungicide at both of these timings. Data collected from this study would indicate that applying fungicide at anthesis timing results in reduced leaf spot control compared to applying at flag leaf stage, however yield remained the same and test weight (kg hL^{-1}) and thousand kernel weight improved when fungicide was applied at anthesis stage and disease pressure was high.

CHAPTER 4

4. Sensitivity of *Pyrenophora tritici-repentis* to Propiconazole and Pyraclostrobin Fungicides

4.1 Introduction

Determining the optimal timing to apply fungicide to best control leaf spots is integral, as applying fungicide at flag leaf stage to control leaf spots and again at anthesis stage to control FHB may lead to insensitivity to a particular fungicide or group of fungicides. If insensitivity caused by overexposure of pathogens to specific fungicide groups does occur, determining where it has occurred and to what extent is paramount in mitigating the spread of these insensitive isolates and developing a strategy to control potential disease outbreaks.

Triazole fungicides have been used to control cereal diseases for over 25 years, with significant use in Europe during the 1980's to control powdery mildew (Jørgensen, 2008). Triazoles have also been used to control septoria leaf blotch in cereals since the 1990's; however, due to several point mutations in the pathogen population, the efficacy of many triazole fungicides such as epoxiconazole and prothioconazole have decreased significantly in several nations including France, The United Kingdom, and Denmark (Clark and Paveley, 2005; Jørgensen and Thygesen 2006).

Strobilurin fungicides function as respiration inhibitors, affecting the Quinone outside site of the mitochondrial gene cytochrome *b* located in complex III (Sierotzki et al., 2007). The first recorded incidence of QoI fungicide insensitivity was in wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) in northern Germany in 1998, two years after they were first introduced (Heaney et al., 2000). Barley powdery mildew (*B. graminis* f. sp. *hordei*) developed insensitivity to strobilurins in northern Germany by 1999 (Heaney et al., 2000). Reports of *M. graminicola* insensitive isolates in the UK and Ireland were made in 2002 (Fraaije et al., 2003). The first

insensitive *P. tritici-repentis* isolates were reported in 2003 (FRAC, 2002). By 2004, insensitivity in *P. tritici-repentis* was observed in field populations leading to the detection of the first QoI insensitivity in *P. tritici-repentis* in middle Sweden (Sierotzki et al., 2007).

There are several amino acid substitutions that have occurred in *P. tritici-repentis* providing varying levels of insensitivity (Fisher and Meunier, 2001). The most common mutation is the substitution of glycine for alanine at amino acid position 143, referred to as G143A and this particular substitution provides the greatest insensitivity to QoI products when compared with other such mutations (Sierotzki et al., 2007). Another mutation, F129L, in which an amino-acid is changed from phenylalanine to leucine at position 129 was also detected, and first reported in populations of *Pyricularia grisea* (Sacc) (Farman 2001). The level of insensitivity conferred by F129L is lower than that provided by G143A (Sierotzki et al., 2007). The F129L mutation was first detected in *P. tritici-repentis* but was rapidly overtaken by the G143A mutation in the population by 2004 (Sierotzki et al., 2007).

Although studies on fungicide insensitivity in *P. tritici-repentis* are limited, insensitivity in this pathogen has been noted (Anon., 2005). Reimann and Deising (2005) collected isolates of *P. tritici-repentis* from fields in the Saxony-Anhalt and Schleswig-Holstein regions of Germany and conducted spore germination assays using epoxiconazole and kresoxim-methyl. They found that isolates collected in Saxony-Anhalt showed no difference in sensitivity based on field fungicide history. The isolates collected in Schleswig-Holstein, however, had a history of repeated fungicide use and ED_{90}s (effective dose at which 90% of spore germination is inhibited) 2-3 times higher than isolates collected in Saxony-Anhalt. Campbell and Crouse (2002) also found that sensitivity to a number of fungicides varied by field, however sensitivity to propiconazole and tebuconazole was similar. Insensitivity to QoI fungicides have been documented in a number of pathogens of

various crops across Europe and Asia including: *Blumeria graminis* f. sp. *tritici* in wheat (Golzar et al., 2016), *Mycosphaerella fijiensis* in banana (Sierotzki et al., 2000), *Plasmopara viticola* in grape (Gisi et al., 2000), *Blumeria graminis* f. sp. *hordei* in barley (Heaney et al. 2000), and *Venturia inaequalis* in apple (Steinfield et al., 2002). Vincelli and Dixon (2001) performed in vitro sensitivity assays on *Pyricularia grisea* isolates collected from fungicide-treated ryegrass in Kentucky and Illinois and found that isolates had become insensitive to QoI fungicides, with over 1,000-fold less sensitivity in comparison to baseline isolates that had never been exposed to fungicides.

It has been reported that the efficacy of QoI fungicides are severely reduced in field populations of *P. tritici-repentis* with high frequencies of G143A isolates, requiring the use of a QoI fungicide mixed with a non-cross-insensitive, single-site fungicide such as a DMI fungicide or multi-site inhibitors such as chlorothalonil to effectively control the disease (Sierotzki et al., 2007). It was discovered in 2007 that 40-50% of *P. tritici-repentis* isolates tested in Denmark were found to have EC_{50} values for strobilurin fungicides of over 10 ppm (Jørgensen 2008). This level of insensitivity to strobilurins is associated with a high frequency of the G143A mutation in the field populations of *P. tritici-repentis*, and as a result triazoles have been mostly recommended in Europe for control of tan spot over strobilurins, due to the latter's ability to control the disease (Jørgensen, 2008).

A loss of sensitivity to QoI fungicides in fungicide-exposed isolates of 100-fold or greater compared to non-exposed baseline samples is often a sign the G143A mutation is present. The G143A mutation has been reported in many different pathogens across Europe, Asia, and North America (Avila-Adame et al., 2003; Brasseur et al., 1996; Brent and Holloman, 2007; Fraaije et al., 2002; Gisi et al., 2002; Ishii et al., 2001; Kianianmomeni et al., 2007; Kim et al., 2003; Wong

et al., 2007). While insensitivity to DMI and carboximide fungicides is only considered a medium risk, pathogens have developed insensitivity to both of these fungicides (Avenot and Michailides, 2007; Luo and Schnabel, 2008). There have also been reports of cross-class insensitivity among isolates, as DMI-insensitive samples of *Monilinia fructicola* became insensitive to azoxystrobin, a QoI fungicide, more rapidly than non DMI-sensitive isolates (Luo and Schnabel, 2008). Reimann and Deising (2004) also found that isolates of *P. tritici-repentis,* once adapted to either DMI or QoI fungicides became cross-insensitive, and would grow on either agar amended with either fungicide or infect leaves treated with either fungicide.

The objective of this study was to determine a baseline sensitivity for isolates of *Pyrenophora tritici-repentis* collected in Saskatchewan and Alberta to propiconazole and pyraclostrobin. This would allow us to determine if reduced sensitivity had occurred based on the EC_{50} values of the isolates, as well as functioning as a baseline to determine if reduced sensitivity to these fungicides occurs in the future.

4.2 Materials and methods

4.2.1 Pathogen isolates and fungicides

The *P. tritici-repentis* isolates examined in this study were collected from across Saskatchewan in 2013 and 2014 and across Alberta in 2010. Twenty-five isolates were collected from Saskatchewan in 2013, 21 from Saskatchewan in 2014 and 25 from Alberta in 2010. After single spore isolation, plugs were stored in 15% glycerol solution at -80°C. Before experiments began, the isolates were revived on potato dextrose agar (PDA) media to ensure viability. The frozen cultures were thawed at room temperature and the plugs plated onto 100 mm plates containing V8-PDA where they were grown in the dark for seven days. Fungicide stock solutions

were made from propiconazole and pyraclostrobin, dissolved in methanol and diluted to 1000x final concentrations.

4.2.2 Spore germination study

For the spore germination experiment, 4 mm mycelial plugs were placed on 100 mm V8-PDA plates and placed in the dark at room temperature for 4-5 days. Plates were then set under light at room temperature for 18-24 hours and then moved to the incubator and placed in the dark for 36 hours at 14 ^{o}C to stimulate sporulation. After sporulation, 1 mL of sterile distilled water was added to the external ring of the fungal colony, where spores were located, and the surface of the colony rubbed gently using a metal loop to suspend the spores in the water. The spore suspension was maintained on ice to avoid spore germination.

At inoculation, 10 µL of each fresh spore suspension was applied to each water agar plate. After 14 hours of incubation in the dark at room temperature, 10 µL of 10x diluted lactophenol was added on top of the culture to inhibit spore germination. For each spot, the total number of both germinated and non-germinated spores was recorded. A spore was considered non-germinated when the germ-tube was shorter than the spore itself. The spore germination rates were evaluated for each isolate and for each fungicide concentration treatment. Three replicates were tested for each isolate, comprised of all concentration treatments plus a control and a methanol control containing only methanol for propiconazole and pyraclostrobin treatments. An additional control was added to the SHAM treatments, treatments containing both SHAM and pyraclostrobin, that contained only SHAM. Data were graphed on a logarithmic scale, with fungicide concentration vs. non-germination rate for each replication. The trend line equation was used to calculate the effective concentration of fungicide that reduced spore germination by 50% compared to the germination rate on non-amended media (EC_{50}). The final fungicide

concentrations for the spore germination experiment for pyraclostrobin were: 0.001, 0.01, 0.1 and 1.0 µg mL^{-1} and for propiconazole were: 50, 100, 200 and 400 µg mL^{-1}. Detailed data are presented in Appendices XXVIII to XLV.

4.2.3 Radial growth study

Radial growth measurements were taken on water agar media at Day 4 and Day 7 using a standard ruler and EC_{50} values, the effective concentration of fungicide that reduced radial growth by 50% was calculated using the formula: *[(radial colony length with any fungicide concentration)/ (radial control colony length)] *100.* The radial growth experiment included 3 replicates and each replicate contained a control with only 5 mL of PDA agar and a methanol control with 5 mL of PDA agar and 5 µL of methanol. Pyraclostrobin was tested using only the fungicide as well as with 5 µL of 100 µg mL^{-1} salicylhydroxamic acid (SHAM), which was dissolved in methanol. Four mm plugs were taken from the 100 mm plates off each isolate and plated onto 60 mm plates containing 5 µL of each of the stock fungicide concentrations and 5 mL of PDA agar. The pyraclostrobin radial growth experiment containing SHAM had an additional control containing 5 mL of PDA and 5 µL of 100 µg mL^{-1} SHAM solution. The final concentrations for propiconazole for use in the radial growth experiment were: 0.05, 0.1, 0.2 and 0.4 µg mL^{-1} and for pyraclostrobin: 0.001, 0.01, 0.1 and 1.0 µg mL^{-1}. Graphs were made on a logarithmic scale with fungicide concentration vs. percentage growth reduction, and the trend line equation used to calculate the EC_{50} value. Graphs were constructed and EC_{50} was calculated in Excel (Microsoft-office). Analysis of variance was conducted using SAS (version 9.3) to study differences among isolates based on EC_{50}. Means were compared using the Tukey test at $P<0.05$. Detailed data are listed in Appendices I to XXVII.

As SHAM had varying effects on radial growth of each isolate tested, ANCOVA was conducted on every isolate at both Day 4 and Day 7 using SAS (version 9.3), with SHAM inhibition (%) used as a covariate of EC_{50} values obtained from each isolate. A graph was generated with LS means values for each isolate. This graph was compared to pyraclostrobin EC_{50} values and pyraclostrobin with SHAM EC_{50} values for both Day 4 and Day 7 to determine whether using SHAM inhibition as a covariate of the fungicide EC_{50} value would result in isolates with similar mean EC_{50} values to isolates exposed only to pyraclostrobin.

4.2.4 Relationship between spore germination, radial growth and fungicide concentration

Regression analysis was conducted using SAS (version 9.3) to determine the relationship between Day 4 and Day 7 radial growth rates of propiconazole, pyraclostrobin and pyraclostrobin with SHAM. Relationships were also determined between spore germination and Day 4 radial growth rate and between germination and Day 7 radial growth rate for each fungicide treatment. Regression analysis was also conducted on propiconazole Day 4 and Day 7 radial growth rates and pyraclostrobin Day 4 and Day 7 radial growth rates to determine if cross-insensitivity existed in any of the isolates for these two fungicide classes. Normality and goodness-of-fit were determined by using the Anderson-Darling test and examining random residual patterns, respectively.

Finally, a model was created to compare pyraclostrobin and pyraclostrobin with SHAM EC_{50} values at Day 4 and Day 7 across four tested concentrations (μg mL^{-1}). The purpose of these tests was to determine if isolates were sensitive to fungicide application without SHAM across all concentrations and whether any patterns existed across concentrations of fungicide in the presence and absence of SHAM.

4.3 Results

4.3.1 Propiconazole treated isolates

The EC_{50} values for spore germination of 70 isolates were calculated for propiconazole (Table 4.1). There were 71 isolates tested, however one of the isolates would not grow on agar and was removed from the spore germination experiment. The EC_{50} values of the isolates varied from 59.36 to 352.33 µg mL^{-1}. There was very little variation in spore germination EC_{50} values. Much higher propiconazole concentrations were required to inhibit germination compared to radial growth of *P. tritici-repentis.* The isolate with the highest EC_{50} value for spore germination was 13SK-TS-58.1, which was significantly higher than the other isolates and may indicate insensitivity to propiconazole.

Table 4.1: The EC_{50} range for spore germination of 70 isolates of *Pyrenophora tritici-repentis* exposed to propiconazole.

Propiconazole concentration (µg mL^{-1})	Number of isolates
0 – 100	34
100 – 200	25
200 – 300	10
300 – 400	1

The EC_{50} values for radial growth of the 71 isolates were calculated at Day 4 and Day 7 for propiconazole sensitivity (Table 4.2). The EC_{50} values varied among isolates from 0.02 to 0.67 µg mL^{-1} on Day 4 and between 0.030 to 1.79 µg mL^{-1} on Day 7.

Table 4.2: The EC_{50} range for radial growth of 71 *Pyrenophora tritici-repentis* isolates exposed to propiconazole and grown on V8-amended potato dextrose agar (PDA) petri plates.

Propiconazole concentration (μg mL^{-1})	EC_{50} Day 4	EC_{50} Day 7
0.0 – 0.1	16	21
0.1 – 0.3	53	47
0.3 – 0.5	1	1
0.5 – 0.7	1	0
>0.7	0	2

The EC_{50} values were plotted for all 71 isolates exposed to propiconazole after 4 days of growth (Fig. 4.1). The only isolate that appeared to be less sensitive than the others was 13Sk-TS-58.1. When looking at the Tukey value, isolate 13SK-TS-58.1 had an EC_{50} value significantly higher than all the other isolates. However, the standard error of the 3 replicates of isolate 13SK-TS-58.1 was very large.

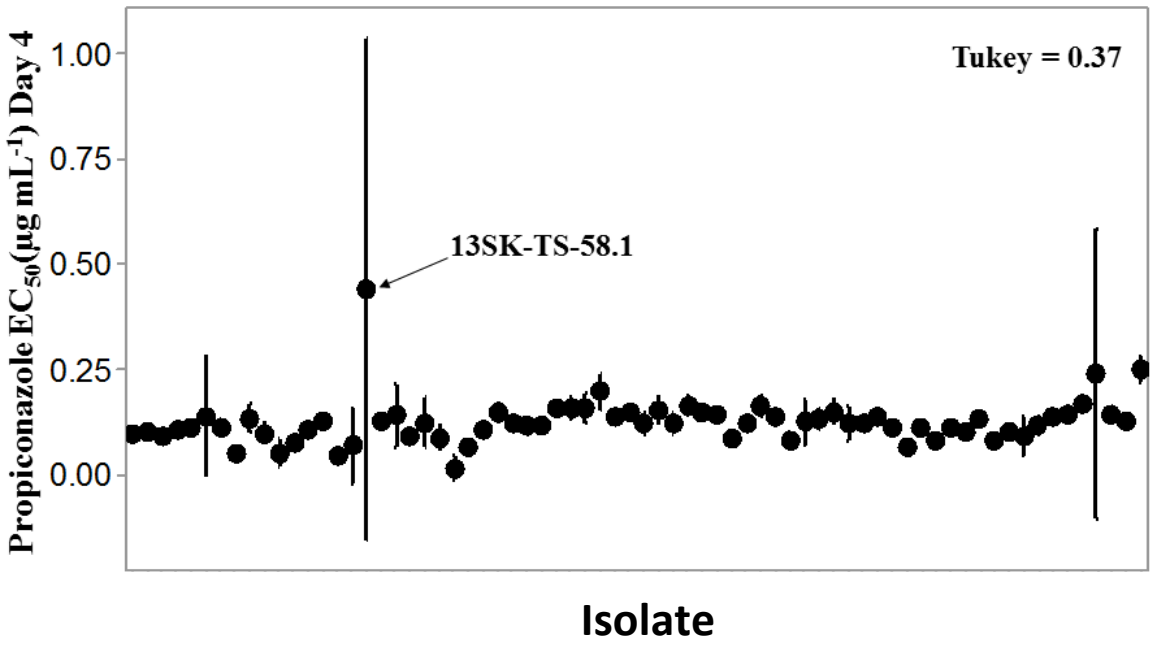

Figure 4.1: Propiconazole EC_{50} (µg mL^{-1}) values for radial mycelial growth of 71 isolates of *Pyrenophora tritici-repentis* at Day 4.

The EC_{50} values were also plotted for all 71 isolates exposed to propiconazole after 7 days of growth (Fig. 4.2). As with Day 4, isolate 13SK-TS-58.1 had an EC_{50} value that was significantly higher than the other isolates, however, the standard error was very large for this isolate at Day 7 as well.

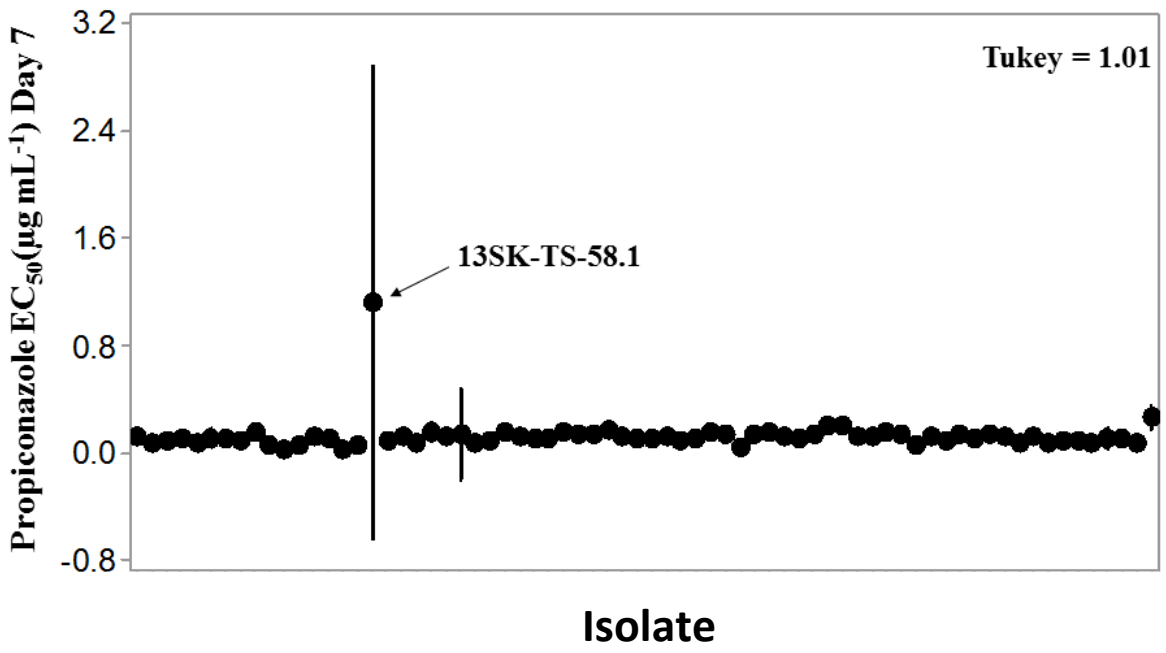

Figure 4.2: Propiconazole EC_{50} (µg mL^{-1}) values for radial mycelial growth of 71 isolates of *Pyrenophora tritici-repentis* at Day 7.

4.3.2 Pyraclostrobin treated isolates

The EC_{50} values for spore germination of the 71 isolates were calculated for pyraclostrobin (Table 4.3). The EC_{50} values ranged from 0.07 to 0.83 µg mL^{-1} among isolates. There was very little variation with the germination EC_{50} values compared to the radial growth EC_{50} values.

Table 4.3: The EC_{50} range for spore germination of 71 isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin.

Pyraclostrobin concentration (µg mL^{-1})	Number of isolates
0 – 0.2	31
0.2 – 0.4	34
0.4 – 0.6	2
0.6 – 0.8	1
0.8 – 1.0	3

The EC_{50} values for radial growth of the 71 isolates were calculated at Day 4 and Day 7 for pyraclostrobin sensitivity (Table 4.4). The EC_{50} values varied among isolates from 0.01 to 2.50 µg mL^{-1} at Day 4 and from 0.02 to 2.99 µg mL^{-1} at Day 7. The distribution of EC_{50} values was very uniform across the spectrum of recorded values of the 71 isolates. Isolate 13SK-TS-29, which had an EC_{50} value of 2.50 µg mL^{-1} was more than 2-fold higher than the isolate that had the next highest EC_{50} value and 9-fold higher than the average EC_{50} value (0.29 µg mL^{-1}) of all 71 isolates measured at Day 4. Isolate 13SK-TS-29 also had the highest EC_{50} value of all 71 isolates tested at Day 7, 2.99 µg mL^{-1} which was 7-fold higher than the average of all isolates tested, 0.41 µg mL^{-1}.

Table 4.4: The EC_{50} range for radial growth of 71 *Pyrenophora tritici-repentis* isolates exposed to pyraclostrobin and grown on V8-amended potato dextrose agar (PDA) petri plates.

Pyraclostrobin concentration (µg mL^{-1})	EC_{50} Day 4	EC_{50} Day 7
0.0 – 0.1	11	14
0.1 – 0.3	41	21
0.3 – 0.5	10	19
0.5 – 0.7	5	6
0.7 – 0.9	1	5
>0.9	3	6

The EC_{50} values were plotted for all 71 isolates exposed to pyraclostrobin after 4 days of growth (Fig. 4.3). The only isolate of the 71 tested that had an EC_{50} value that would suggest the isolate was less sensitive to pyraclostrobin than the other tested isolates was 13SK-TS-29. Based on the Tukey test, isolate 13SK-TS-29 had a significantly greater EC_{50} value than all other isolates except AB70-1. However, the isolate had a very high standard error value and the lowest EC_{50} value for the isolate was close to the EC_{50} values of the other isolates.

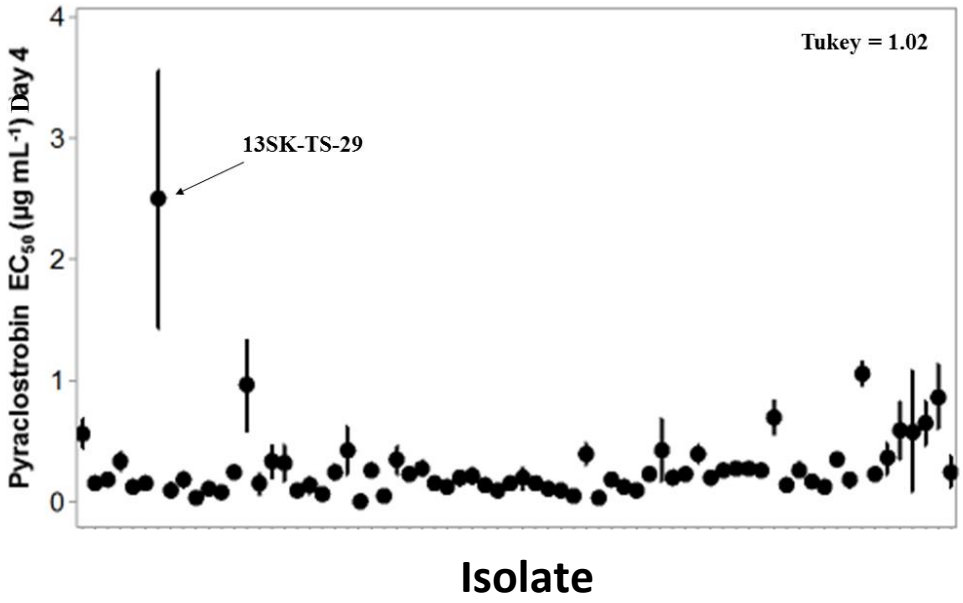

Figure 4.3: Pyraclostrobin EC_{50} (µg mL^{-1}) values for raidial mycelial growth of 71 isolates of *Pyrenophora tritici-repentis* at Day 4.

The EC_{50} values were plotted for all 71 isolates exposed to pyraclostrobin after 7 days of growth (Fig. 4.4). Isolate 13SK-TS-29 had an EC_{50} value greater than all other isolates except AB70-1. Isolate AB70-1, however, was not greater than the other isolates. Isolate 13SK-TS-29 did have a very high standard error value and the lowest EC_{50} value for the isolate was close to the EC_{50} values of the other isolates.

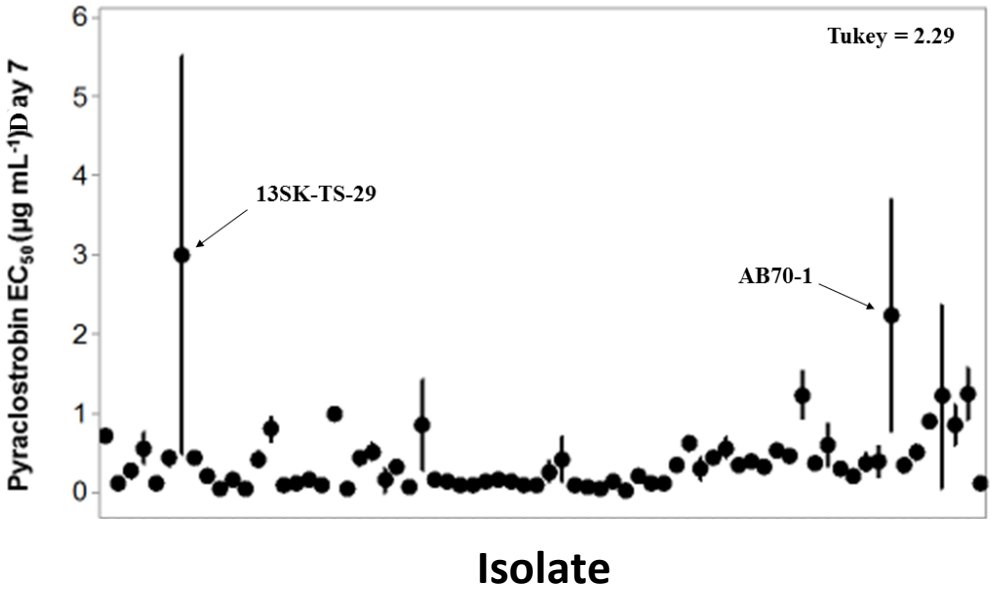

Figure 4.4: Pyraclostrobin EC_{50} (µg mL^{-1}) values for radial mycelial growth of 71 isolates of *Pyrenophora tritici-repentis* at Day 7.

The EC_{50} values for spore germination of the 71 isolates collected were calculated for pyraclostrobin with SHAM (Table 4.5). The EC_{50} values of the isolates varied from 0.00001 to 0.00809 µg mL^{-1}. There was very little variation in spore germination EC_{50} values compared to the radial growth EC_{50} values.

Table 4.5: The EC_{50} range for spore germination of 71 isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM.

Pyraclostrobin with SHAM concentration (µg mL^{-1})	Number of isolates
0 - 0.00009	2
0.00009 - 0.0009	25
0.0009 - 0.009	44

The EC_{50} values for radial growth of the 71 isolates collected were calculated at Day 4 and Day 7 for pyraclostrobin and SHAM sensitivity (Table 4.6). The EC_{50} values for the 71 isolates exposed to pyraclostrobin with SHAM was highly variable and was most likely due to the fact that

SHAM on its own inhibited radial growth among the isolates by 17.0 to 66.5% on Day 4 and 13.9% to 63.9% on Day 7.

Table 4.6: The EC_{50} range for radial growth of 71 *Pyrenophora tritici-repentis* isolates exposed to pyraclostrobin with SHAM and grown on V8-amended potato dextrose agar (PDA) petri plates.

Pyraclostrobin with SHAM concentration (μg mL^{-1})	EC_{50} Day 4	EC_{50} Day 7
0.0 – 0.0001	2	3
0.0001 – 0.001	6	5
0.001 – 0.01	25	25
0.01 – 0.1	38	33
0.1 – 1.0	0	5

When EC_{50} values were plotted for the 71 isolates exposed to pyraclostrobin with SHAM after 4 days of growth, there were two isolates that appeared to be less sensitive to pyraclostrobin with SHAM than the others, AB49-1 and AB51-3 (Fig. 4.5). These two isolates were 4- to 8-fold less sensitive to pyraclostrobin with SHAM than the majority of the other isolates. When looking at the Tukey value however, the EC_{50} values of these two isolates were not significantly different than 19 of the other isolates tested. Similar to the other isolates in previous treatments that possessed greater EC_{50} values compared to the other isolates, AB49-1 and AB51-3 had very high standard errors.

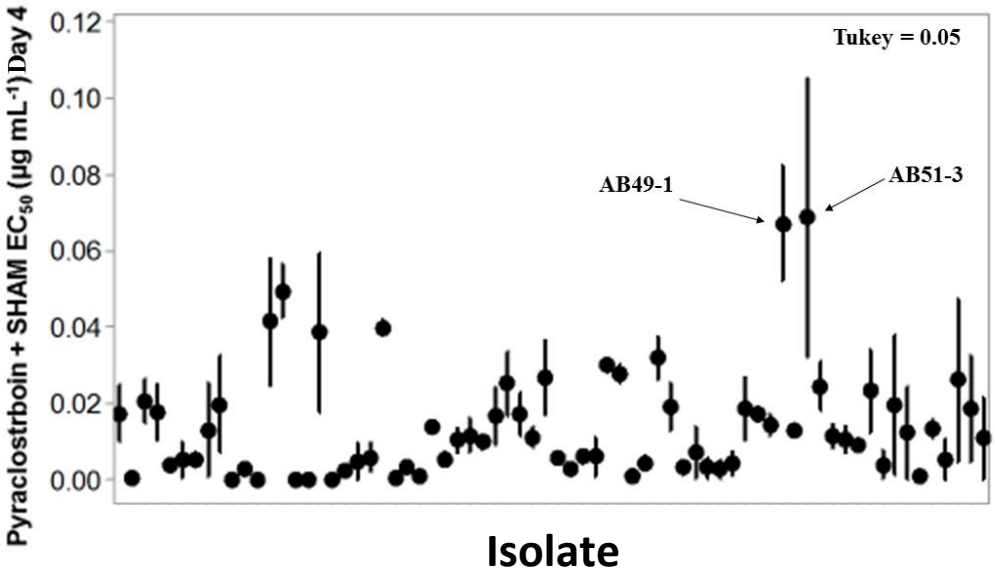

Figure 4.5: Pyraclostrobin with SHAM EC_{50} (µg mL^{-1}) values for radial mycelial growth of 71 isolates of *Pyrenophora tritici-repentis* at Day 4.

A discrepancy in isolate sensitivity was noted when isolates were exposed to pyraclostrobin with SHAM after 4 days of growth compared to when they were exposed to pyraclostrobin alone. Using ANCOVA (analysis of covariance), SHAM inhibition (%) was set as a covariate of pyraclostrobin with SHAM to determine if differences between isolate sensitivity to pyraclostrobin with SHAM and pyraclostrobin alone were due to differences in SHAM inhibition (%) between the isolates. The results showed that SHAM inhibition had no significant effect on the model. When the SHAM treatment was used as a covariate, the LSMEAN values of isolates exposed to pyraclostrobin with SHAM were the same as the plotted EC_{50} values in the previous model. Isolates AB49-1 and AB51-3 remained less sensitive compared with the other isolates (Fig.4.6).

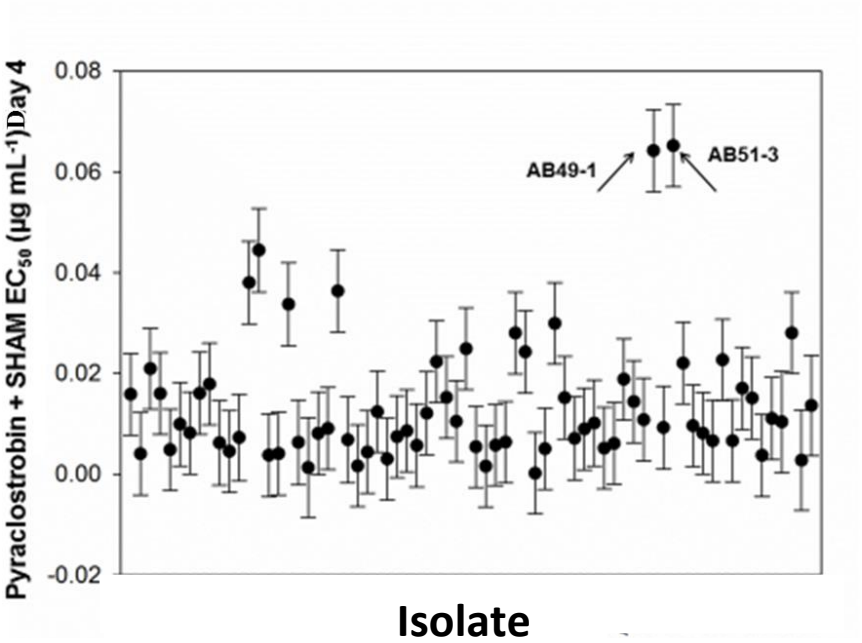

Figure 4.6: Pyraclostrobin with SHAM EC_{50} (µg mL^{-1}) LSMEAN values for radial mycelial growth for 71 isolates of *Pyrenophora tritici-repentis* at Day 4 from ANCOVA analysis.

When EC_{50} values were plotted for the 71 isolates exposed to pyraclostrobin with SHAM after 7 days of growth, there were three isolates that appeared to be less sensitive to pyraclostrobin with SHAM than the others, AB49-1, AB51-3 and AB56-2 (Fig. 4.7). These three isolates were 4- to 8-fold less sensitive to pyraclostrobin with SHAM than the majority of the other isolates. Looking at the Tukey value, these three isolates had EC_{50} values that were similar to three other isolates: AB73-3, 13SK-TS-54 and 13SK-TS-69. Only AB49-1 and AB51-3 had EC_{50} values greater than the majority of the other isolates. These three isolates also had very high standard error.

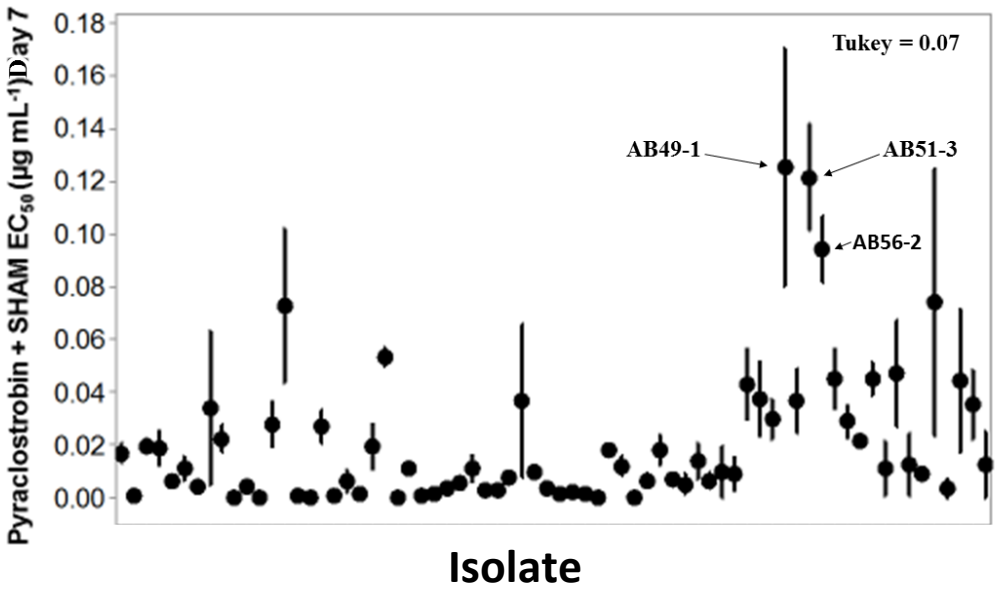

Figure 4.7: Pyraclostrobin with SHAM EC_{50} (µg mL^{-1}) values for radial mycelial growth of 71 isolates of *Pyrenophora tritici-repentis* at Day 7.

A discrepancy in isolate sensitivity was noted when isolates were exposed to pyraclostrobin with SHAM after 7 days of growth compared to when they were exposed to pyraclostrobin alone. Using ANCOVA, SHAM inhibition (%) was set as a covariate of pyraclostrobin with SHAM to determine if differences between isolate sensitivity to pyraclostrobin with SHAM and pyraclostrobin alone were due to differences in SHAM inhibition (%) between the isolates. The results showed that, as with the mean EC_{50} values of isolates exposed to pyraclostrobin with SHAM, LSMEAN values of isolates exposed to pyraclostrobin with SHAM were very similar, with isolates AB49-1, AB51-3 and AB56-2 less sensitive compared to the other isolates (Fig. 4.8).

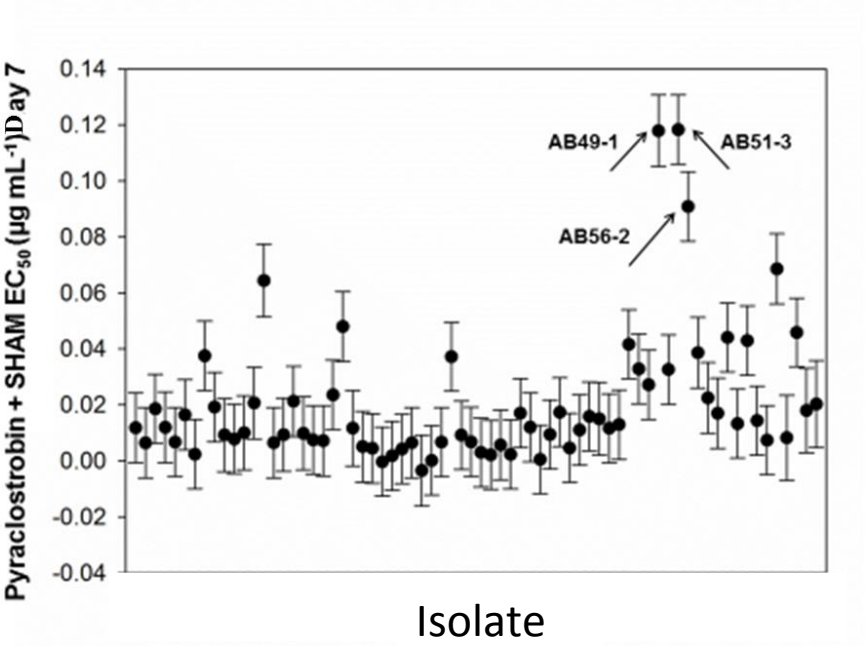

Figure 4.8: Pyraclostrobin with SHAM EC_{50} (µg mL^{-1}) LSMEAN values for radial mycelial growth for 71 isolates of *Pyrenophora tritici-repentis* at Day 7 from ANCOVA analysis.

Regression analysis was conducted using SAS (version 9.3) to explore the relationship between radial growth measurements of isolates exposed to propiconazole across four concentrations at 4 and 7 days of growth, as well as between pyraclostrobin at 4 and 7 days of growth and pyraclostrobin with SHAM at 4 and 7 days of growth. The results comparing propiconazole radial growth at Day 4 and Day 7 indicated that there was a statistically significant relationship between the radial growth measurements, however the relationship was moderate, with an R-squared value of 0.40 (Fig. 4.9). There was a stronger relationship between radial growth at Day 4 and Day 7 for isolates exposed to pyraclostrobin, as well as pyraclostrobin and SHAM, with R-squared values of 0.73 and 0.66, respectively (Table 4.7).

Table 4.7: Regression analysis of radial growth of *Pyrenophora tritici-repentis* at Day 4 and Day 7 treated with propiconazole, pyraclostrobin and pyraclostrobin with SHAM.

Regression analysis	*P*-value	R^2
Propiconazole radial growth Day 4 vs. Day 7	<0.0001	0.40
Pyraclostrobin radial growth Day 4 vs. Day 7	<0.0001	0.73
Pyraclostrobin and SHAM radial growth Day 4 vs. Day 7	<0.0001	0.66

Correlation analysis was conducted using SAS (version 9.3) to explore the relationship between propiconazole and pyraclostrobin radial growth at Day 4 and Day 7, as well as between propiconazole germination and radial growth at Day 4 and Day 7, pyraclostrobin germination and radial growth at Day 4 and Day 7, and pyraclostrobin with SHAM germination and radial growth at Day 4 and Day 7. The results of the correlation analysis showed that there was no relationship between propiconazole and pyraclostrobin radial growth at either Day 4 or Day 7. There was a strong correlation between propiconazole germination and radial growth at Day 4 with an R value of 0.69 and a weak correlation between propiconazole germination and radial growth at Day 7 with an r value of 0.33. There was a weak relationship between pyraclostrobin germination and radial growth at Day 4 and Day 7 with R values of 0.32 and 0.36, respectively. There was a moderately strong and strong relationship between pyraclostrobin germination and radial growth at Day 4 and Day 7 with R values of 0.58 and 0.6.

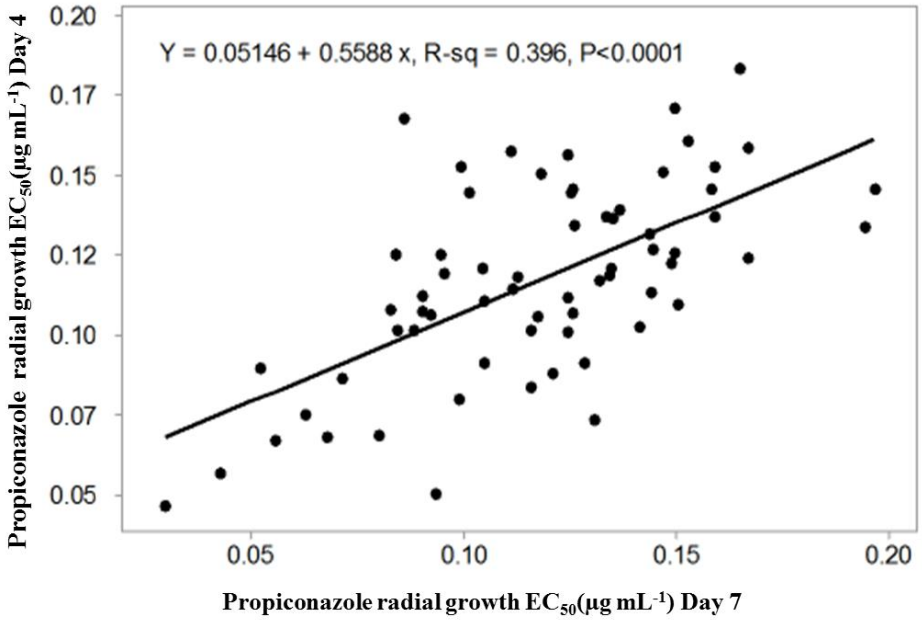

Figure 4.9: Regression analysis of propiconazole radial growth of *Pyrenophora tritici-repentis* at Day 4 and Day 7.

Table 4.8: Correlation analysis of spore germination and radial growth of *Pyrenophora tritici-repentis* treated with propiconazole, pyraclostrobin and pyraclostrobin with SHAM.

Correlation analysis	*P*-value	R
Propiconazole vs. pyraclostrobin Day 4 radial growth	<0.8440	0
Propiconazole vs. pyraclostrobin Day 7 radial growth	<0.6160	0.20
Propiconazole germination vs. radial growth Day 4	<0.0001	0.69
Propiconazole germination vs. radial growth Day 7	<0.0050	0.33
Pyraclostrobin germination vs. radial growth Day 4	<0.0050	0.32
Pyraclostrobin germination vs. radial growth Day 7	<0.0020	0.36
Pyraclostrobin and SHAM germination vs radial growth Day 4	<0.0001	0.58
Pyraclostrobin and SHAM germination vs radial growth Day 7	<0.0001	0.60

The results of the relative mycelial growth comparisons between treatments of pyraclostrobin and pyraclostrobin with SHAM (Fig. 4.11 and 4.12) suggested that pyraclostrobin is effective at reducing radial growth of *P. tritici-repentis* isolates even in the absence of SHAM.

However, the introduction of SHAM resulted in a 20-fold lower mean EC_{50} value at both Day 4 and Day 7 than pyraclostrobin alone.

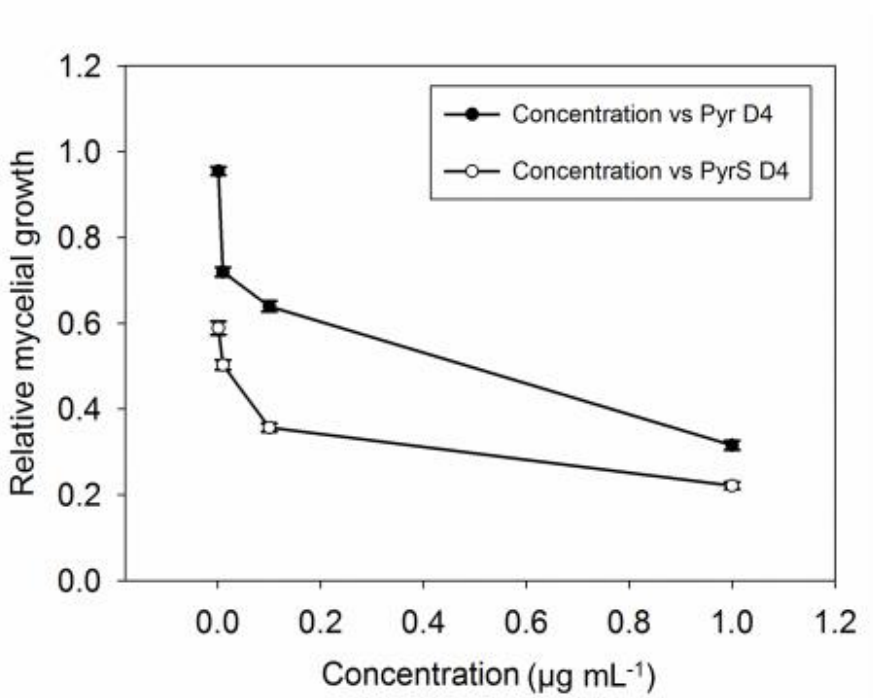

Figure 4.11: Relative mycelial growth comparison at several fungicide concentrations between 71 isolates of *Pyrenophora tritici-repentis* treated with pyraclostrobin (Pyr) and pyraclostrobin with SHAM (PyrS) at Day 4.

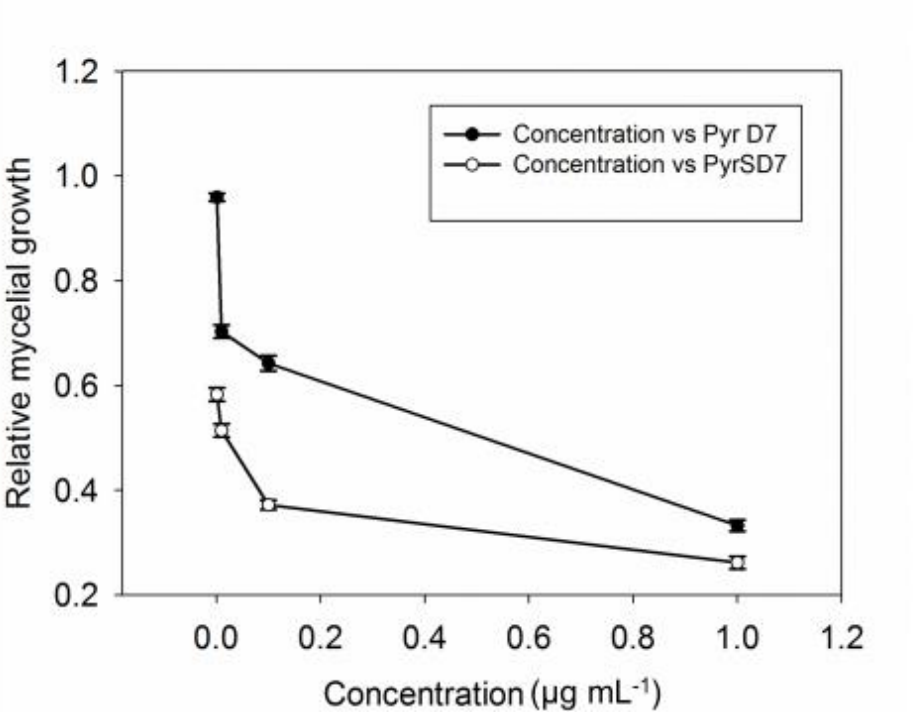

Figure 4.12: Relative mycelial growth comparison at several fungicide concentrations between 71 isolates of *Pyrenophora tritici-repentis* treated with pyraclostrobin (Pyr) and pyraclostrobin with SHAM (PyrS) at Day 7.

4.4 Discussion

The EC_{50} values for spore germination of isolates of *P. tritici-repentis* exposed to propiconazole were all very similar, with less than a 10-fold difference between the highest and lowest EC_{50} value of the 71 isolates. For isolates exposed to pyraclostrobin, EC_{50} values were also very similar, with 64 of the 71 isolates having EC_{50} values between 0.1 and 1.0 µg mL^{-1}. When comparing propiconazole to pyraclostrobin, pyraclostrobin was on average 200 times more effective at a given concentration at inhibiting *P. tritici-repentis* conidial germination.

Adding SHAM to pyraclostrobin reduced EC_{50} values on average 100-fold. The EC_{50} values for pyraclostrobin with SHAM, however, had greater variability amongst the isolates compared to pyraclostrobin alone. The SHAM on its own had no effect on spore germination; this coupled with the increase in isolate sensitivity when SHAM was added to pyraclostrobin would suggest that spores of *P. tritici-repentis* were able to circumvent the effect of fungicide itself to

some extent by the activation of the alternative respiration pathway, the alternative oxidase (AOX) pathway. Patel et al. (2012) exposed 136 isolates of *P. tritici-repentis* collected from North Dakota from 2007-2009 to pyraclostrobin with SHAM and found EC_{50} values between 0.0013 to 0.0027 µg mL^{-1} with a mean value of 0.0017 µg mL^{-1}. These values were in line with what was observed in this study, however, the range of values in this study were much greater, which may be because the isolates were collected over a wider geographic area and had greater genetic diversity. Sierotzki et al. (2007) also conducted a baseline sensitivity study on isolates of *P. tritici-repentis* from Europe and found EC_{50} values for azoxystrobin ranging from 0.007 to 0.73 µg mL^{-1} with isolates carrying the F129L and G143A mutations much less sensitive (EC_{50} values from 0.08 to 100 µg mL^{-1}). These EC_{50} values were much higher than what was observed in this study and may be because azoxystrobin was less effective than pyraclostrobin to control *P. tritici-repentis* or due to the fact that strobilurins, particularly azoxystrobin, has been heavily used in Europe to control leaf spot diseases. Reduced sensitivity or outright insensitivity have been documented in several countries.

The EC_{50} values for radial growth measurements of all three fungicide treatments varied to a greater extent when compared to EC_{50} values for spore germination. The majority of radial growth EC_{50} values for propiconazole were between 0 and 0.3 µg mL^{-1}, with 68 of 71 isolates on Day 4 and Day 7 between these concentrations. There was a 20-fold difference among the 71 isolates on Day 4 and a 20-fold difference on Day 7. Isolate 13SK-TS-58.1 was less sensitive to propiconazole compared with other isolates as it had a 5-fold higher tolerance (EC_{50} = 0.67 µg mL^{-1}) than the mean sensitivity of the 71 isolates tested on Day 4 (EC_{50} = 0.14 µg mL^{-1}), and an 11-fold higher tolerance (EC_{50} = 1.79 µg mL^{-1}) on Day 7 (EC_{50} = 0.16 µg mL^{-1}). However, other isolates had higher tolerance to propiconazole as well, such as 14y2, which had an EC_{50} value of

0.58 µg mL^{-1} on Day 4 and 1.20 µg mL^{-1} on Day 7, 4 and 7-fold higher than the mean sensitivity of the 71 isolates, 0.14 and 0.16 µg mL^{-1} on Day 4 and Day 7, respectively. The EC_{50} values obtained in this study were similar to those obtained by Beard et al. (2009) who tested sensitivity of 50 isolates of *P. tritici-repentis* collected in Western Australia, 18 of which were collected in 1986-1987 and 32 collected from 2001-2003. They determined the sensitivity of these isolates to propiconazole, epoxiconazole and tebuconazole and recorded mean EC_{50} values of 0.39, 0.19 and 0.25 µg mL^{-1}, respectively. These values are in line with, albeit slightly higher than EC_{50} values obtained in this study. Hunger and Brown (1987) tested the effects of propiconazole and tebuconazole on 10 isolates of *P. tritici-repentis* from Oklahoma and Texas and found mean EC_{50} values of 0.04 µg mL^{-1} for propiconazole and 0.19 µg mL^{-1} for tebuconazole. The mean EC_{50} value for tebuconazole was similar to the mean EC_{50} value of propiconazole found in this study, however the mean EC_{50} value for propiconazole was 4-fold lower. This may be due in part because Hunger and Brown (1987) used formulated fungicide rather than unformulated technical grade fungicide that was used in this study and in the study by Beard et al. (2009). The formulation additives present in the formulated fungicide may have affected EC_{50} values.

The EC_{50} values for *P. tritici-repentis* isolates exposed to pyraclostrobin were within the range of 0.0013 to 2.50 µg mL^{-1} on Day 4 and 0.024 to 2.99 µg mL^{-1} on Day 7 with the majority of isolates on Day 4 (61) and on Day 7 (53) between 0 and 0.5 µg mL^{-1}. There was a greater variation among isolates of *P. tritici-repentis* for pyraclostrobin sensitivity than propiconazole sensitivity, which varied by 1862-fold on Day 4 and 125-fold on Day 7. One isolate (13SK-TS-29) had a higher EC_{50} value on Day 4 and Day 7 compared to the other isolates. The EC_{50} value was 2.50 µg mL^{-1} on Day 4, 9-fold higher than the mean sensitivity of the 71 isolates, 2.99 µg mL^{-1} on Day 7, 7-fold higher than the mean sensitivity of isolates tested. This isolate was very fast

growing over the first 4 days of growth which may explain the relatively high EC_{50} value. By Day 7, the growth of this isolate had begun to slow and the difference between this isolate and other isolates became less pronounced.

There was no data on baseline fungicide sensitivity of *P. tritici-repentis* in Saskatchewan to compare to, however studies were conducted in North Dakota in 2000 on another pathogen, *Pyricularia grisea* (Kim et al., 2003). This pathogen, like *P. tritici-repentis*, was known to include isolates possessing the G143A mutation. The G143A mutation in *P. grisea* conferred a 1,300-fold reduction in sensitivity to azoxystrobin compared to the baseline EC_{50} value of isolates collected previously and a 1,000-fold reduced sensitivity to trifloxystrobin. Another mutation, F129L, also appears in both pathogens and confers a lower level of insensitivity to fungicides, a 140-fold reduction in sensitivity to azoxystrobin and a 30-fold reduction in sensitivity to trifloxystrobin (Kim et al., 2003). All of the isolates in my study appeared to have varying degrees of sensitivity to pyraclostrobin with none possessing insensitivity to pyraclostrobin great enough to be caused by either mutation.

There was a 3.2×10^{11}-fold difference in radial growth EC_{50} values for pyraclostrobin with SHAM between the most sensitive ($EC_{50} = 2.1 \times 10^{-13}$ µg mL^{-1}) and least sensitive ($EC_{50} = 0.069$ µg mL^{-1}) isolate of *P. tritici-repentis* on Day 4, and an 11,385-fold difference between the most sensitive ($EC_{50} = 1.1 \times 10^{-5}$ µg mL^{-1}) and least sensitive ($EC_{50} = 0.13$ µg mL^{-1}) isolate on Day 7. However, two isolates at Day 4 and three isolates at Day 7 were very sensitive to pyraclostrobin with SHAM, and SHAM on its own inhibited radial growth of these isolates by more than 60%. Eliminating these isolates, there was still a greater than 1000-fold difference in sensitivity between the most sensitive and the least sensitive isolates on both Day 4 and Day 7. The high variation in isolate sensitivity to pyraclostrobin with SHAM may be due to variable levels of sensitivity to

SHAM, as SHAM alone reduced radial growth from 13% to 63% depending on the isolate. There appeared to be a wide variation of sensitivity among isolates to pyraclostrobin and as such, it was unlikely that insensitivity due to fungicide exposure had occurred. Overall pyraclostrobin with SHAM reduced radial growth 20 times more effectively on Day 4 and Day 7 compared to pyraclostrobin alone.

The regression analyses of radial growth of isolates on propiconazole amended media revealed a moderately weak relationship between EC_{50} values taken at Day 4 and Day 7 (R^2 = 0.40), and a moderately strong relationship for EC_{50} values of isolates on pyraclostrobin amended media between Day 4 and Day 7 (R^2 = 0.73). For pyraclostrobin with SHAM, there was a strong relationship between the log of Day 4 and Day 7 EC_{50} values (R^2 = 0.66). Given these strong relationships between Day 4 and Day 7 for the various fungicide treatments, it would be possible to use Day 4 EC_{50} values to calculate Day 7 EC_{50} values. However, it may be better to measure radial growth twice, despite the time saved by calculating radial growth EC_{50} values based on one timing. Calculating radial growth EC_{50} values twice would also control for isolates that are very fast or very slow growing.

The EC_{50} values for radial growth of isolates on propiconazole amended media were correlated to pyraclostrobin radial growth EC_{50} values at both Day 4 and Day 7. There was no correlation at Day 4 (R = 0) and the correlation was very low at Day 7 (R = 0.2); there appeared to be no correlation between sensitivity to propiconazole and sensitivity to pyraclostrobin. This result was expected as strobilurins and azoles target pathogens in two very different ways and it would be unusual for exposure to one mode of action to affect the sensitivity of a completely different class of fungicide. Spore germination EC_{50} values were also correlated to radial growth EC_{50} values for all three fungicide treatments at both Day 4 and Day 7. In the majority of instances there was

a weak correlation suggesting that using germination EC_{50} values to predict radial growth EC_{50} values would be difficult. This may be because strobilurins are generally more effective at controlling conidial germination, while azoles are largely inefficient at controlling conidial germination and are much more effective at limiting mycelial growth.

4.5 Conclusion

Based on the results of this study, it would appear that reduced sensitivity of *P. tritici-repentis* to either azole or strobilurin fungicides has not occurred in Saskatchewan or Alberta. From the radial growth and germination studies, it would appear that assessing spore germination is a more accurate method of measuring isolate sensitivity to strobilurins as the concentration of pyraclostrobin required to inhibit 50% of the conidial germination was much lower compared with the concentration required to inhibit 50% of the radial growth. The SHAM also had no effect on conidial germination in the absence of fungicide, making it easier to obtain more accurate results. With respect to azoles, it would appear that measuring radial growth would be a more accurate way to determine fungicide sensitivity as very large concentrations of propiconazole were required to inhibit conidial germination. From the regression analyses, it would be best to continue measuring radial growth for at least two days to accurately determine EC_{50} values and to compensate for fast or slow growing isolates. It was also determined that correlation between conidial and mycelial fungicide sensitivity was sporadic, and it would be difficult to justify using spore germination inhibition to predict mycelial growth rate inhibition and vice-versa. This study has determined a baseline for strobilurin and azole sensitivity for *P. tritici-repentis* in Saskatchewan and Alberta. Repeated studies should be conducted, however, to determine the long term effects of applying these fungicides to fields across the prairies.

CHAPTER 5

5. General discussion and conclusion

5.1 General discussion

Canada is one of the world's major spring wheat producers. However, yield losses due to disease outbreaks can severely limit production. Leaf spots and fusarium head blight (FHB) are two diseases that commonly occur in spring wheat grown in Saskatchewan and the Prairie region, however, using fungicide to control these diseases usually means applying fungicide twice, once at flag leaf stage (ZGS39) to control leaf spots and again at anthesis stage (ZGS60) to control FHB. This practice may be cost prohibitive and could also lead to the development of insensitivity to commonly used fungicides such as quinone outside inhibitors (QoIs) and demethylation inhibitors (DMIs) in pathogens known for developing insensitivity through mutations like the G143A and F129L mutation, such as *Pyrenophora tritici-repentis*, the causal agent of tan spot of wheat.

This project focused on fungicide timing, to determine the optimal time to apply fungicide to adequately control leaf spots. It was determined that applying fungicide at anthesis stage, when growers normally spray for FHB, adequately controlled leaf spots compared to applying at flag leaf stage. It was shown that leaf spot disease incidence was higher when fungicide was applied at anthesis compared to flag leaf stage but yield was not significantly affected. Fusarium head blight levels remained high in Melfort despite fungicide application at both timings. This may be because the fungicide was applied five days later than the optimum timing at Melfort and fusarium may have already been established reducing effectiveness in supressing the disease.

Applying fungicide at both timings provided a slightly higher yield when disease was high, but was not economically viable as yield only increased by 105 kg ha^{-1} compared to a single application at anthesis stage, and by 136 kg ha^{-1} compared with a single application at flag leaf

stage. Prothioconazole + tebuconazole costs \$42.24 ha^{-1} and tebuconazole \$30.26 ha^{-1}, with an additional \$37.00 ha^{-1} to apply the fungicide. With a wheat price of \$194.72 per tonne, a second application of prothioconazole + tebuconazole would need to produce a 407 kg ha^{-1} increase in yield and a second application of tebuconazole would need to produce an additional 345 kg ha^{-1} to recoup the cost of application. Applying the same fungicide family twice per growing season may also lead to fungicide insensitive pathogen. It was noted by Chang et al. (2007) that applying pyraclostrobin several times each year led to the selection of insensitive populations, while also contributing to the reduction in efficacy of pyraclostrobin to control ascochyta blight. Ahmed et al. (2014) reported that isolates of *Didymella rabiei* in chickpea collected in 2007 were 190 times less sensitive to pyraclostrobin than isolates collected in 2003.

There was also no difference in yield when a half-rate or full-rate of fungicide was applied in either the high disease or low disease locations. However, applying only a half-rate of fungicide may result in greater selection for insensitive pathogen populations. Applying a full-rate of fungicide is recommended to reduce the risk of fungicide insensitive pathogen populations as it reduces selection pressure by ensuring that all or nearly all of the population is killed (Waard 1993).

The second project focused on *Pyrenophora tritici-repentis* sensitivity to two fungicides, the quinone outside inhibitor, pyraclostrobin and the demethylation inhibitor, propiconazole. Increased exposure to the same fungicide group has been shown to cause insensitivity in several pathogen species, as natural selection allows for isolates possessing insensitivity due to mutations, such as the G143A and F129L mutation to survive and reproduce, conferring increased insensitivity to the pathogen population as a whole. This process is hastened in fungicides that target a single-site, such as the quinone outside inhibitors. In this study, it was determined that

there was a moderate relationship between conidial and mycelial insensitivity to propiconazole and pyraclostrobin. However, the relationship between spore germination inhibition and radial growth inhibition varied greatly among isolates of *P. tritici-repentis*. As such, it would be advisable to conduct both radial growth inhibition and spore germination inhibition studies to gain a more in-depth understanding of pathogen population sensitivity to fungicides. Correlation studies were also conducted to determine the relationship between propiconazole insensitivity and pyraclostrobin insensitivity in the pathogen population. It appeared from the correlation analysis that no cross-resistance relationship existed in the population of *P. tritici-repentis* with respect to these two fungicides. Similar studies have also been conducted looking at the relationship between cross-resistance of QoI and DMI fungicides in *Venturia inaequalis* (apple scab), *Alternaria spp.* (alternaria blight of pistachio), *Mycosphaerella graminicola* (septoria leaf blotch) and *Cercospora beticola* (sugar beet leaf spot). In all of these pathogens, no example of cross-resistance between QoI and DMI fungicides could be found (Karaoglandis and Bardas 2006; Küng-Färber et al., 2002; Ma et al., 2003; Mavroeidi and Shaw 2005). There was however, a report that cross-resistance occurred between QoI and DMI fungicides in *Uncinula necator* (grapevine powdery mildew), although only partial insensitivity was observed (Wong and Wilcox 2002). It has been suggested however that insensitivity development in one fungicide class may lead to accelerated insensitivity to another unrelated class (Köller and Wilcox 2000, 2001).

A baseline sensitivity was developed for isolates collected from Saskatchewan in 2013-2014 and Alberta in 2010. None of the isolates tested displayed reduced sensitivity to either propiconazole or pyraclostrobin based on the calculated radial growth and spore germination EC_{50} values. However, several isolates showed slightly reduced sensitivity to either propiconazole or

pyraclostrobin, although these values were not outside the range of what would be expected normal variation within the pathogen population.

5.2 Future work

In the future, the fungicide application timing experiment could be expanded to include more varieties of spring wheat to determine if tall, dwarf and semi-dwarf varieties all have the same optimal fungicide timing to control leaf spots, as some of the shorter varieties may have leaf spots reach the flag leaf earlier resulting in reduced yield if fungicide is applied at a later timing when leaf spots have already established.

With regards to the fungicide sensitivity experiment, several other classes of QoI and DMI fungicides could be tested besides the strobilurins and triazoles, respectively. It would also be beneficial to send the isolates with slightly lower sensitivity to the QoI fungicides for testing to determine whether the G143A or F129L mutations were present.

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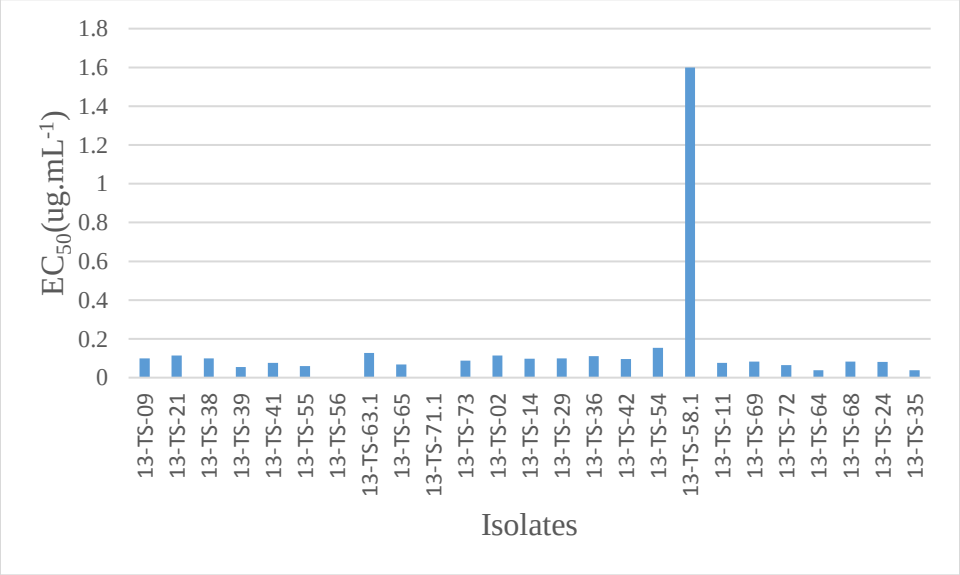
APPENDICES

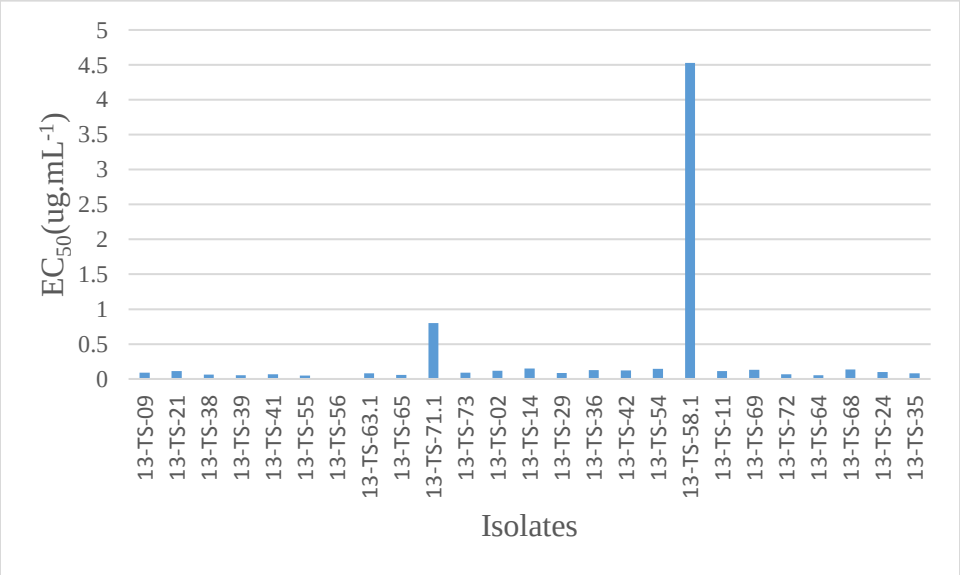
Appendix I: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Saskatchewan in 2013.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7(µg mL^{-1})
13-TS-09	Propiconazole	0.10001	0.09092
13-TS-21	Propiconazole	0.11387	0.11552
13-TS-38	Propiconazole	0.1	0.063
13-TS-39	Propiconazole	0.05496	0.05453
13-TS-41	Propiconazole	0.07578	0.0673
13-TS-55	Propiconazole	0.05946	0.04764
13-TS-56	Propiconazole	0.00010	0.00133
13-TS-63.1	Propiconazole	0.12690	0.08134
13-TS-65	Propiconazole	0.06870	0.05682
13-TS-71.1	Propiconazole	NA	0.80035
13-TS-73	Propiconazole	0.08816	0.08870
13-TS-02	Propiconazole	0.11448	0.11892
13-TS-14	Propiconazole	0.09756	0.14955
13-TS-29	Propiconazole	0.10000	0.08727
13-TS-36	Propiconazole	0.11164	0.12877
13-TS-42	Propiconazole	0.09632	0.12439
13-TS-54	Propiconazole	0.15301	0.14549
13-TS-58.1	Propiconazole	1.59996	4.52569
13-TS-11	Propiconazole	0.07647	0.11249
13-TS-69	Propiconazole	0.08260	0.13348
13-TS-72	Propiconazole	0.06503	0.06961
13-TS-64	Propiconazole	0.03789	0.05359
13-TS-68	Propiconazole	0.08298	0.13629
13-TS-24	Propiconazole	0.08164	0.10000
13-TS-35	Propiconazole	0.03855	0.07937

Appendix II: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to propiconazole from Saskatchewan in 2013.



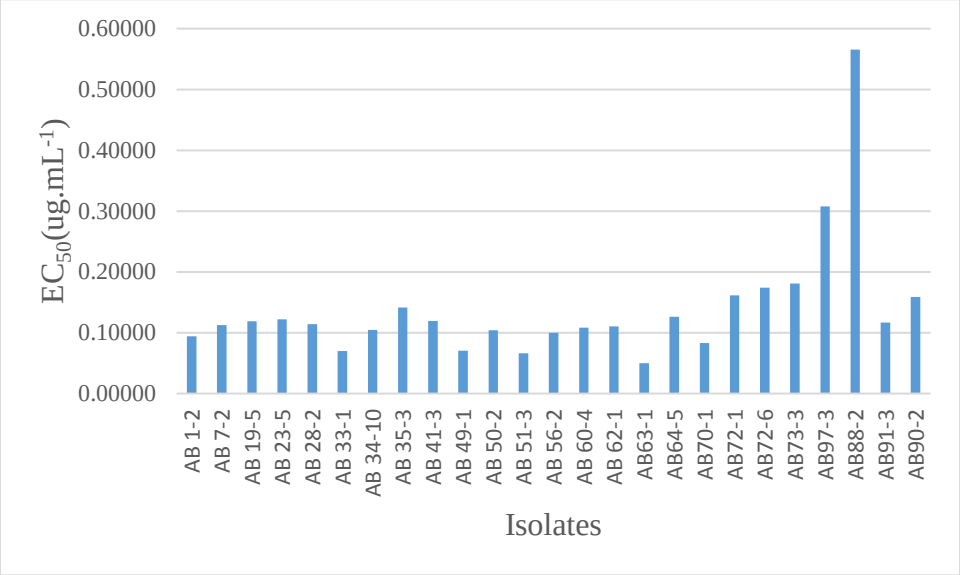
Appendix III: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 7 exposed to propiconazole from Saskatchewan in 2013.



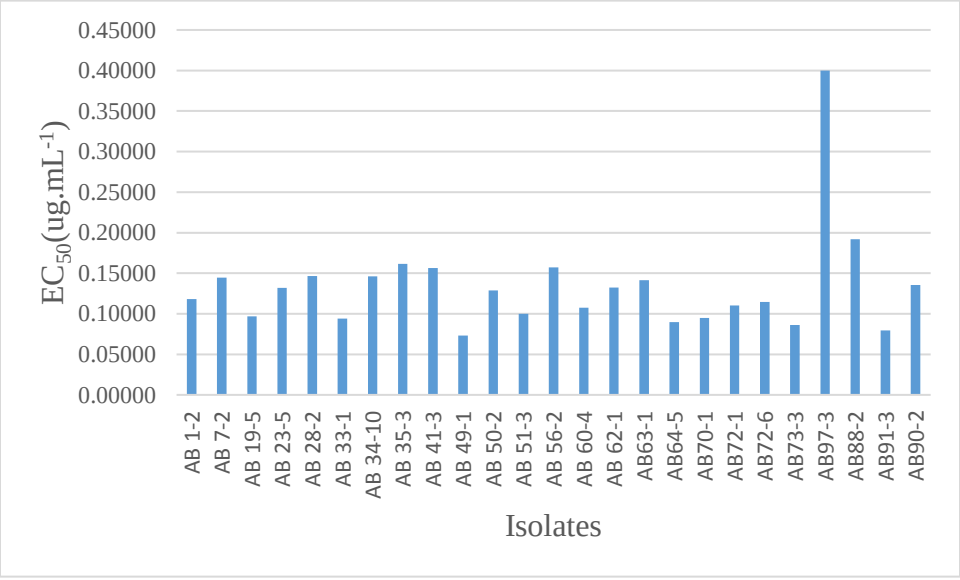
Appendix IV: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Alberta in 2010.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7(µg mL^{-1})
AB 1-2	Propiconazole	0.09406	0.11821
AB 7-2	Propiconazole	0.11280	0.14447
AB 19-5	Propiconazole	0.11894	0.09702
AB 23-5	Propiconazole	0.12190	0.13195
AB 28-2	Propiconazole	0.11388	0.14674
AB 33-1	Propiconazole	0.06981	0.09427
AB 34-10	Propiconazole	0.10490	0.14611
AB 35-3	Propiconazole	0.14142	0.16159
AB 41-3	Propiconazole	0.11959	0.15659
AB 49-1	Propiconazole	0.07071	0.07308
AB 50-2	Propiconazole	0.10416	0.12866
AB 51-3	Propiconazole	0.06626	0.10000
AB 56-2	Propiconazole	0.10001	0.15725
AB 60-4	Propiconazole	0.10833	0.10757
AB 62-1	Propiconazole	0.11041	0.13230
AB63-1	Propiconazole	0.05001	0.14143
AB64-5	Propiconazole	0.12599	0.08963
AB70-1	Propiconazole	0.08298	0.09481
AB72-1	Propiconazole	0.16159	0.11041
AB72-6	Propiconazole	0.17412	0.11448
AB73-3	Propiconazole	0.18114	0.08620
AB97-3	Propiconazole	0.30752	0.40000
AB88-2	Propiconazole	0.56567	0.19199
AB91-3	Propiconazole	0.11665	0.07938
AB90-2	Propiconazole	0.15873	0.13557

Appendix V: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to propiconazole from Alberta in 2010.



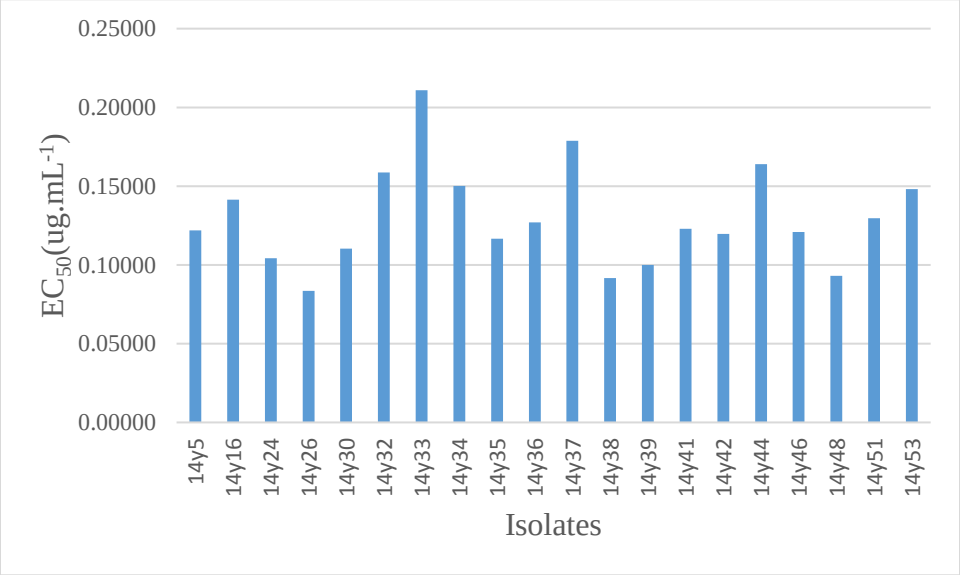
Appendix VI: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 7 exposed to propiconazole from Alberta in 2010.



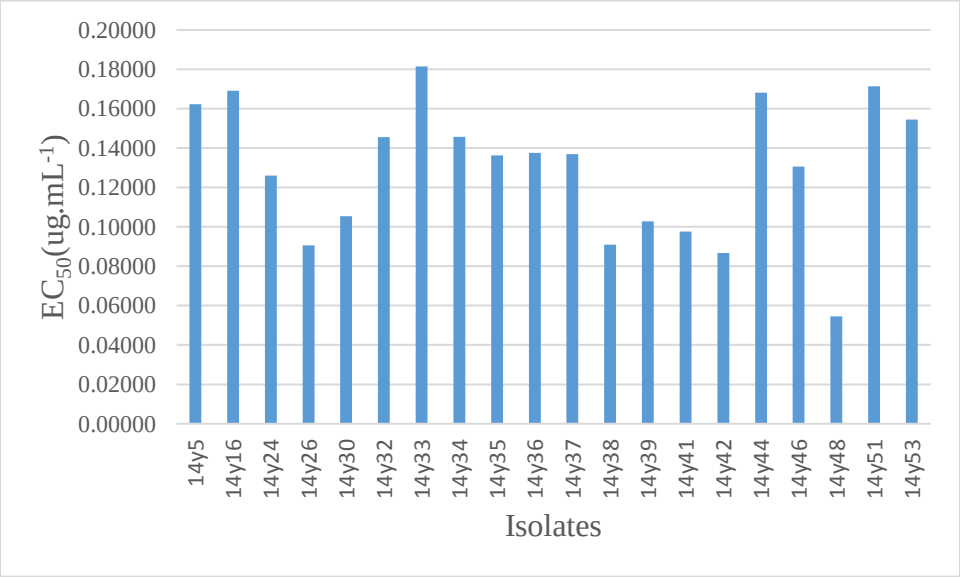
Appendix VII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Saskatchewan in 2014.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7 (µg mL^{-1})
14y2	Propiconazole	NA	NA
14y5	Propiconazole	0.12190	0.16227
14y16	Propiconazole	0.14142	0.16918
14y24	Propiconazole	0.10416	0.12599
14y26	Propiconazole	0.08346	0.09057
14y30	Propiconazole	0.11039	0.10537
14y32	Propiconazole	0.15873	0.14549
14y33	Propiconazole	0.21096	0.18144
14y34	Propiconazole	0.15014	0.14578
14y35	Propiconazole	0.11665	0.13630
14y36	Propiconazole	0.12690	0.13758
14y37	Propiconazole	0.17874	0.13687
14y38	Propiconazole	0.09170	0.09090
14y39	Propiconazole	0.10001	0.10287
14y41	Propiconazole	0.12289	0.09756
14y42	Propiconazole	0.11959	0.08674
14y44	Propiconazole	0.16406	0.16819
14y46	Propiconazole	0.12081	0.13056
14y48	Propiconazole	0.09308	0.05452
14y51	Propiconazole	0.12969	0.17144
14y53	Propiconazole	0.14821	0.15445

Appendix VIII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to propiconazole from Saskatchewan in 2014.



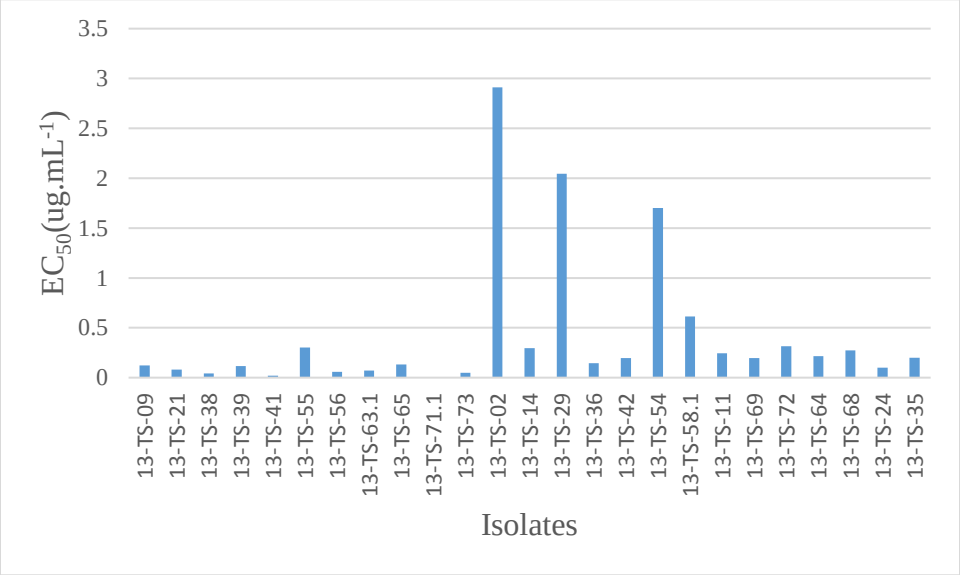
Appendix IX: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 7 exposed to propiconazole from Saskatchewan in 2014.



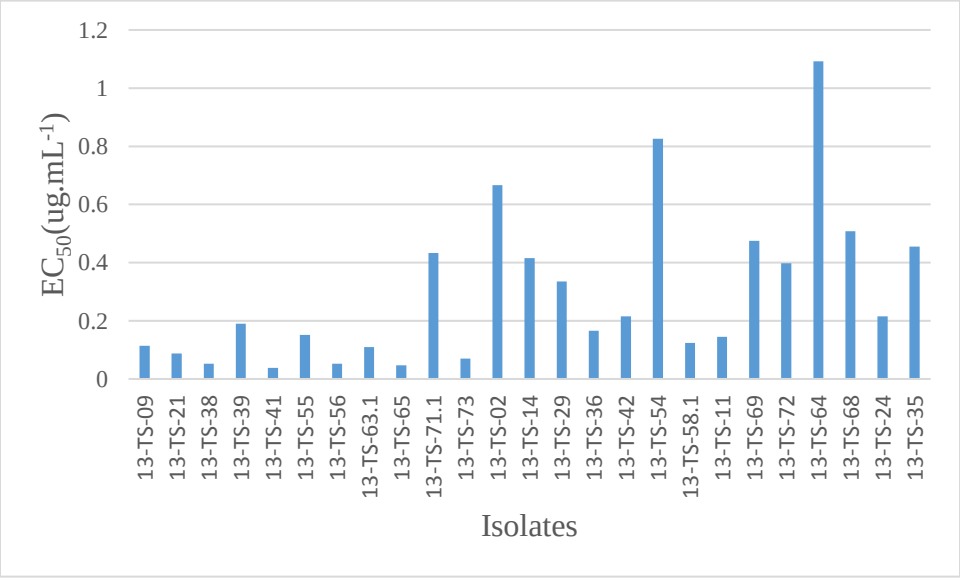
Appendix X: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Saskatchewan in 2013.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7 (µg mL^{-1})
13-TS-09	Pyraclostrobin	0.12114	0.11366
13-TS-21	Pyraclostrobin	0.08072	0.08799
13-TS-38	Pyraclostrobin	0.04108	0.05217
13-TS-39	Pyraclostrobin	0.11514	0.18957
13-TS-41	Pyraclostrobin	0.02116	0.03856
13-TS-55	Pyraclostrobin	0.30076	0.15200
13-TS-56	Pyraclostrobin	0.05994	0.05282
13-TS-63.1	Pyraclostrobin	0.07197	0.10996
13-TS-65	Pyraclostrobin	0.13335	0.04725
13-TS-71.1	Pyraclostrobin	NA	0.43287
13-TS-73	Pyraclostrobin	0.04924	0.06968
13-TS-02	Pyraclostrobin	2.91260	0.66608
13-TS-14	Pyraclostrobin	0.29470	0.41590
13-TS-29	Pyraclostrobin	2.04335	0.33546
13-TS-36	Pyraclostrobin	0.14385	0.16566
13-TS-42	Pyraclostrobin	0.19745	0.21544
13-TS-54	Pyraclostrobin	1.70116	0.82541
13-TS-58.1	Pyraclostrobin	0.61267	0.12362
13-TS-11	Pyraclostrobin	0.24605	0.14485
13-TS-69	Pyraclostrobin	0.19619	0.47476
13-TS-72	Pyraclostrobin	0.31623	0.39812
13-TS-64	Pyraclostrobin	0.21545	1.09259
13-TS-68	Pyraclostrobin	0.27384	0.50799
13-TS-24	Pyraclostrobin	0.09999	0.21544
13-TS-35	Pyraclostrobin	0.19953	0.45555

Appendix XI: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to pyraclostrobin from Saskatchewan in 2013.



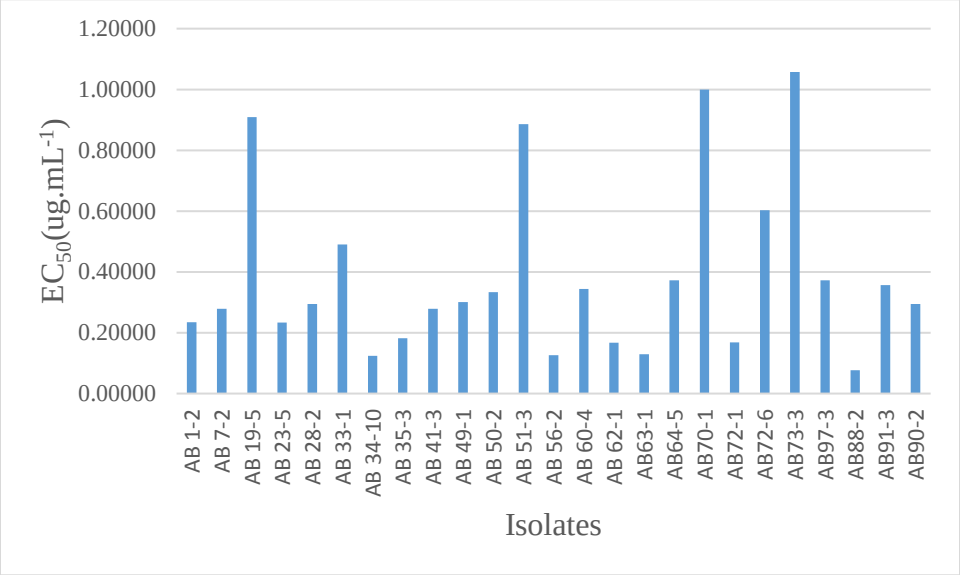
Appendix XII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 7 exposed to pyraclostrobin from Saskatchewan in 2013.



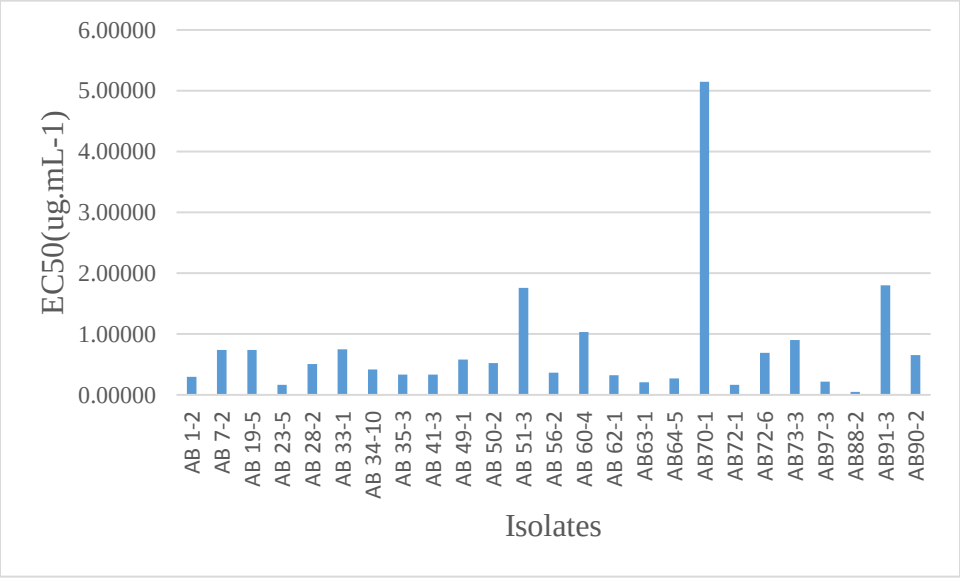
Appendix XIII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Alberta in 2010.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7 (µg mL^{-1})
AB 1-2	Pyraclostrobin	0.23418	0.29895
AB 7-2	Pyraclostrobin	0.27825	0.73967
AB 19-5	Pyraclostrobin	0.90855	0.73968
AB 23-5	Pyraclostrobin	0.23301	0.16534
AB 28-2	Pyraclostrobin	0.29472	0.50801
AB 33-1	Pyraclostrobin	0.49032	0.74990
AB 34-10	Pyraclostrobin	0.12451	0.41752
AB 35-3	Pyraclostrobin	0.18233	0.33546
AB 41-3	Pyraclostrobin	0.27824	0.33545
AB 49-1	Pyraclostrobin	0.30077	0.58268
AB 50-2	Pyraclostrobin	0.33323	0.52234
AB 51-3	Pyraclostrobin	0.88581	1.75899
AB 56-2	Pyraclostrobin	0.12589	0.36579
AB 60-4	Pyraclostrobin	0.34404	1.03553
AB 62-1	Pyraclostrobin	0.16682	0.32502
AB63-1	Pyraclostrobin	0.12979	0.20664
AB64-5	Pyraclostrobin	0.37278	0.27147
AB70-1	Pyraclostrobin	1.00000	5.14692
AB72-1	Pyraclostrobin	0.16819	0.16535
AB72-6	Pyraclostrobin	0.60324	0.69275
AB73-3	Pyraclostrobin	1.05777	0.90200
AB97-3	Pyraclostrobin	0.37278	0.21542
AB88-2	Pyraclostrobin	0.07667	0.04771
AB91-3	Pyraclostrobin	0.35699	1.80013
AB90-2	Pyraclostrobin	0.29472	0.65432

Appendix XIV: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to pyraclostrobin from Alberta in 2010.



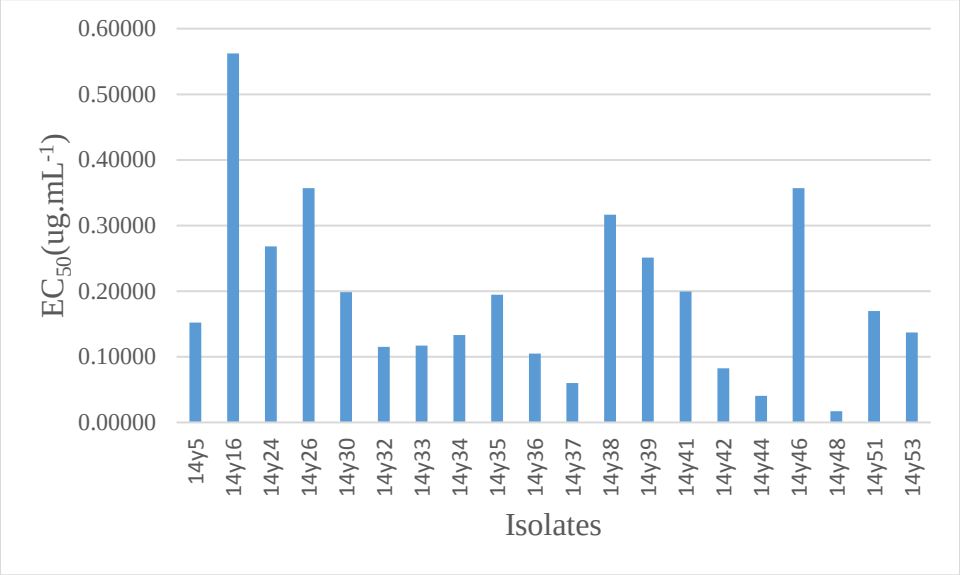
Appendix XV: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 7 exposed to pyraclostrobin from Alberta in 2010.



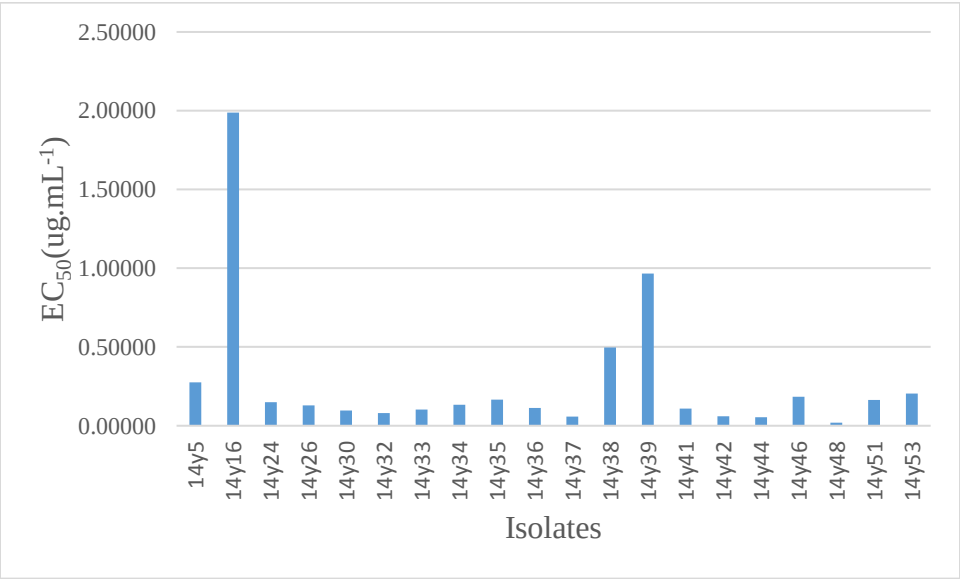
Appendix XVI: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Saskatchewan in 2014.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7 (µg mL^{-1})
14y2	Pyraclostrobin	NA	NA
14y5	Pyraclostrobin	0.15199	0.27641
14y16	Pyraclostrobin	0.56237	1.98734
14y24	Pyraclostrobin	0.26826	0.14986
14y26	Pyraclostrobin	0.35698	0.12875
14y30	Pyraclostrobin	0.19856	0.09753
14y32	Pyraclostrobin	0.11513	0.07966
14y33	Pyraclostrobin	0.11700	0.10281
14y34	Pyraclostrobin	0.13335	0.13335
14y35	Pyraclostrobin	0.19474	0.16566
14y36	Pyraclostrobin	0.10491	0.11364
14y37	Pyraclostrobin	0.05995	0.05834
14y38	Pyraclostrobin	0.31623	0.49622
14y39	Pyraclostrobin	0.25117	0.96715
14y41	Pyraclostrobin	0.19953	0.10807
14y42	Pyraclostrobin	0.08254	0.05995
14y44	Pyraclostrobin	0.04061	0.05402
14y46	Pyraclostrobin	0.35699	0.18360
14y48	Pyraclostrobin	0.01714	0.02031
14y51	Pyraclostrobin	0.16950	0.16379
14y53	Pyraclostrobin	0.13717	0.20534

Appendix XVII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to pyraclostrobin from Saskatchewan in 2014.



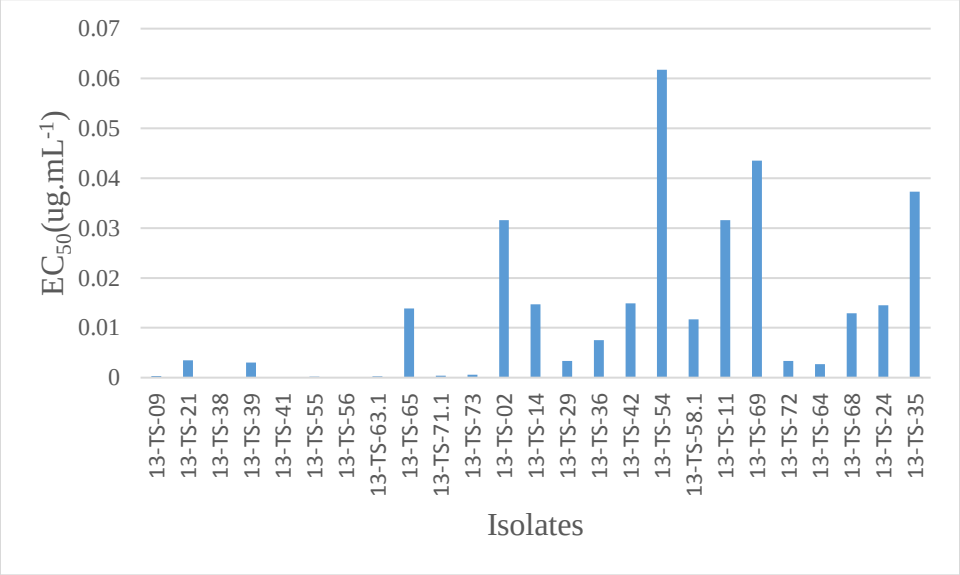
Appendix XIII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 7 exposed to pyraclostrobin from Saskatchewan in 2014.



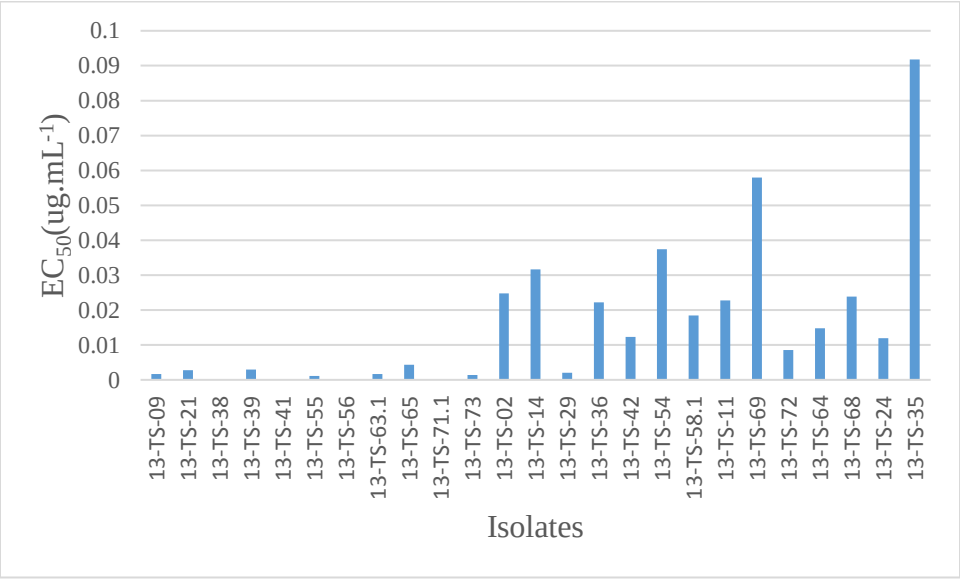
Appendix XIX: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Saskatchewan in 2013.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7 (µg mL^{-1})
13-TS-09	Pyr+SHAM	0.00032	0.00167
13-TS-21	Pyr+SHAM	0.00346	0.00278
13-TS-38	Pyr+SHAM	2.15e^-5	0.00007
13-TS-39	Pyr+SHAM	0.00303	0.00296
13-TS-41	Pyr+SHAM	4.64e^-13	0.00002
13-TS-55	Pyr+SHAM	0.00019	0.00110
13-TS-56	Pyr+SHAM	0.00002	0.00021
13-TS-63.1	Pyr+SHAM	0.00026	0.00168
13-TS-65	Pyr+SHAM	0.01389	0.00434
13-TS-71.1	Pyr+SHAM	0.00042	0.00000
13-TS-73	Pyr+SHAM	0.00059	0.00142
13-TS-02	Pyr+SHAM	0.03162	0.02479
13-TS-14	Pyr+SHAM	0.01468	0.03162
13-TS-29	Pyr+SHAM	0.00332	0.00207
13-TS-36	Pyr+SHAM	0.00750	0.02223
13-TS-42	Pyr+SHAM	0.01492	0.01233
13-TS-54	Pyr+SHAM	0.06176	0.03746
13-TS-58.1	Pyr+SHAM	0.01172	0.01848
13-TS-11	Pyr+SHAM	0.03162	0.02276
13-TS-69	Pyr+SHAM	0.04354	0.05796
13-TS-72	Pyr+SHAM	0.00332	0.00855
13-TS-64	Pyr+SHAM	0.00268	0.01478
13-TS-68	Pyr+SHAM	0.01292	0.02388
13-TS-24	Pyr+SHAM	0.01453	0.01190
13-TS-35	Pyr+SHAM	0.03728	0.09183

Appendix XX: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to pyraclostrobin with SHAM from Saskatchewan in 2013.



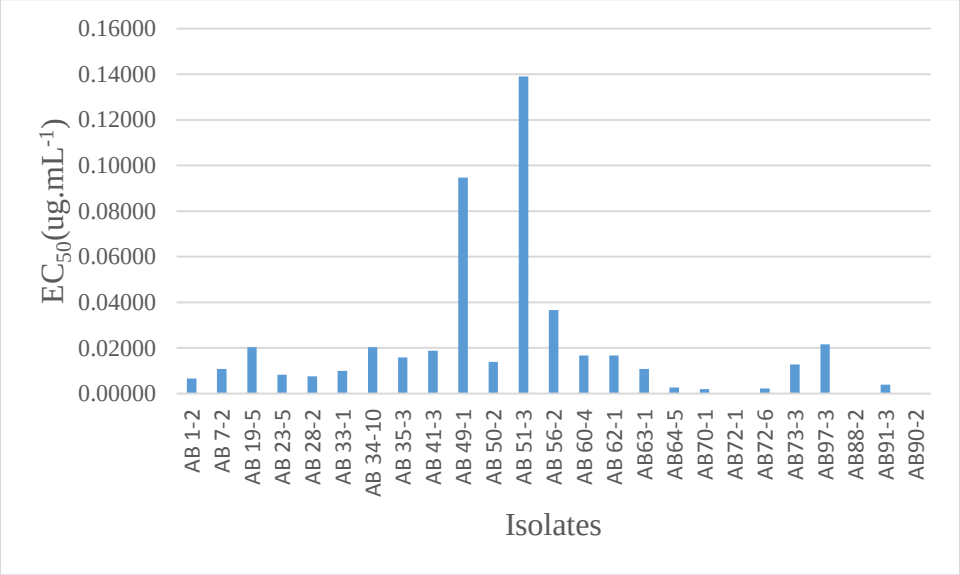
Appendix XXI: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to pyraclostrobin with SHAM from Saskatchewan in 2013.



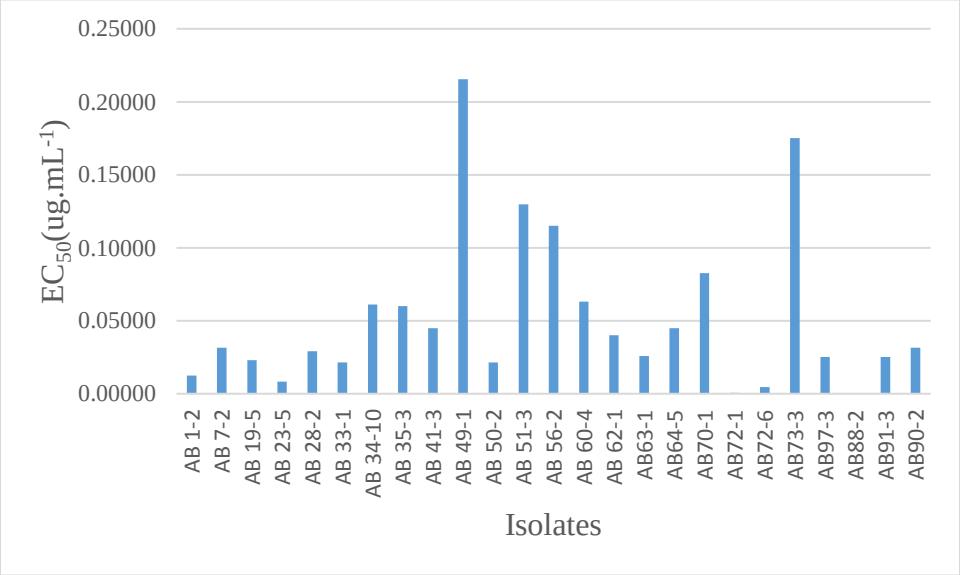
Appendix XXII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Alberta in 2010.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7 (µg mL^{-1})
AB 1-2	Pyr+SHAM	0.00658	0.01243
AB 7-2	Pyr+SHAM	0.01075	0.03162
AB 19-5	Pyr+SHAM	0.02031	0.02297
AB 23-5	Pyr+SHAM	0.00822	0.00822
AB 28-2	Pyr+SHAM	0.00750	0.02916
AB 33-1	Pyr+SHAM	0.01000	0.02136
AB 34-10	Pyr+SHAM	0.02031	0.06122
AB 35-3	Pyr+SHAM	0.01585	0.05995
AB 41-3	Pyr+SHAM	0.01874	0.04498
AB 49-1	Pyr+SHAM	0.09467	0.21545
AB 50-2	Pyr+SHAM	0.01389	0.02155
AB 51-3	Pyr+SHAM	0.13896	0.12978
AB 56-2	Pyr+SHAM	0.03665	0.11513
AB 60-4	Pyr+SHAM	0.01668	0.06310
AB 62-1	Pyr+SHAM	0.01668	0.04019
AB63-1	Pyr+SHAM	0.01075	0.02575
AB64-5	Pyr+SHAM	0.00268	0.04499
AB70-1	Pyr+SHAM	0.00202	0.08255
AB72-1	Pyr+SHAM	0.000003	0.00060
AB72-6	Pyr+SHAM	0.00222	0.00464
AB73-3	Pyr+SHAM	0.01274	0.17509
AB97-3	Pyr+SHAM	0.02154	0.02512
AB88-2	Pyr+SHAM	0.00010	9.9e^-70
AB91-3	Pyr+SHAM	0.00398	0.02512
AB90-2	Pyr+SHAM	0.00010	0.03162

Appendix XXIII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to pyraclostrobin with SHAM from Alberta in 2010.



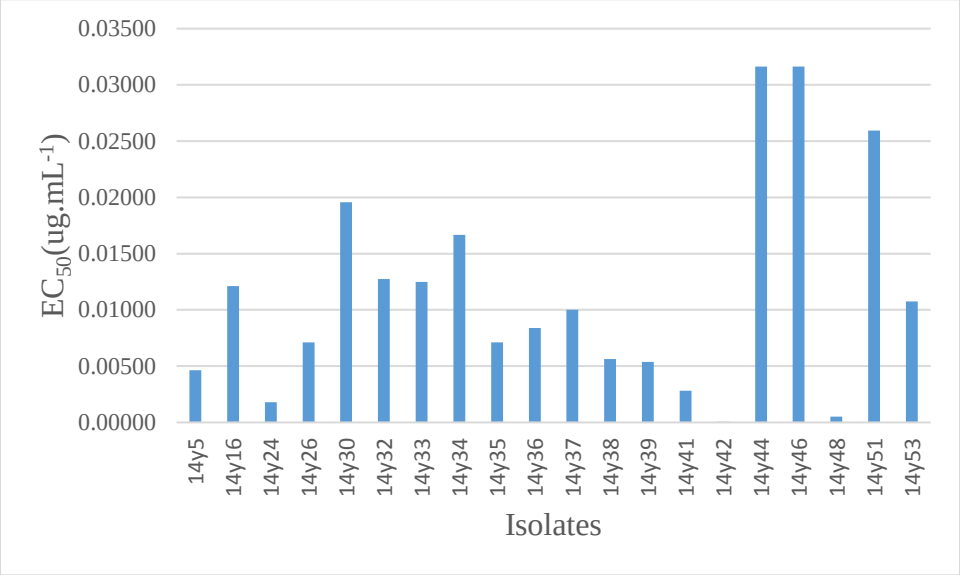
Appendix XXIV: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 7 exposed to pyraclostrobin with SHAM from Alberta in 2010.



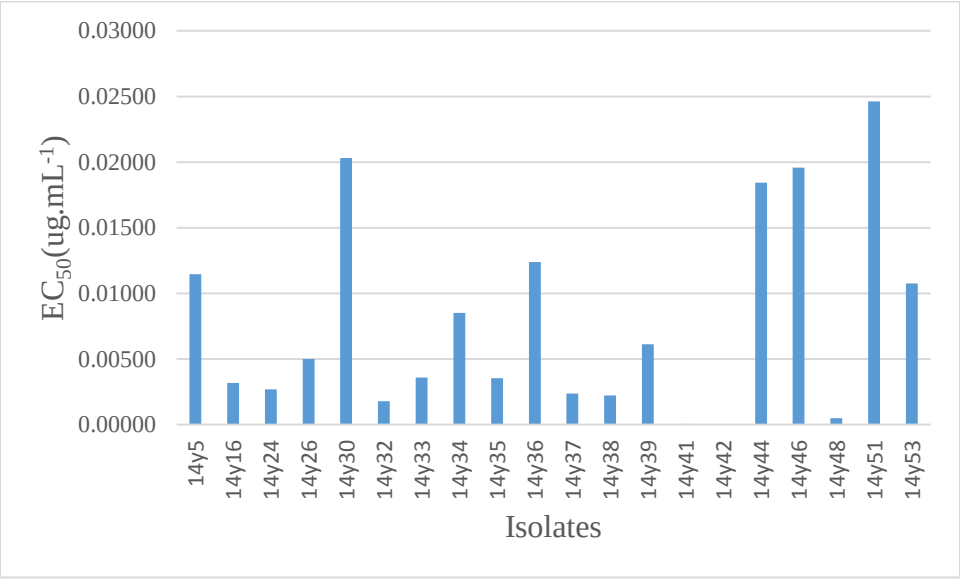
Appendix XXV: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Saskatchewan in 2014.

Isolate	Fungicide	EC_{50} Day 4 (µg mL^{-1})	EC_{50} Day 7 (µg mL^{-1})
14y2	Pyr+SHAM	NA	NA
14y5	Pyr+SHAM	0.00464	0.01145
14y16	Pyr+SHAM	0.01211	0.00316
14y24	Pyr+SHAM	0.00178	0.00268
14y26	Pyr+SHAM	0.00711	0.00501
14y30	Pyr+SHAM	0.01957	0.02031
14y32	Pyr+SHAM	0.01274	0.00178
14y33	Pyr+SHAM	0.01250	0.00359
14y34	Pyr+SHAM	0.01668	0.00851
14y35	Pyr+SHAM	0.00711	0.00353
14y36	Pyr+SHAM	0.00838	0.01239
14y37	Pyr+SHAM	0.01000	0.00237
14y38	Pyr+SHAM	0.00562	0.00222
14y39	Pyr+SHAM	0.00538	0.00611
14y41	Pyr+SHAM	0.00283	0.00006
14y42	Pyr+SHAM	0.00005	4.159e^-7
14y44	Pyr+SHAM	0.03162	0.01843
14y46	Pyr+SHAM	0.03162	0.01957
14y48	Pyr+SHAM	0.00052	0.00048
14y51	Pyr+SHAM	0.02593	0.02462
14y53	Pyr+SHAM	0.01075	0.01075

Appendix XXVI: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 4 exposed to pyraclostrobin with SHAM from Saskatchewan in 2014.



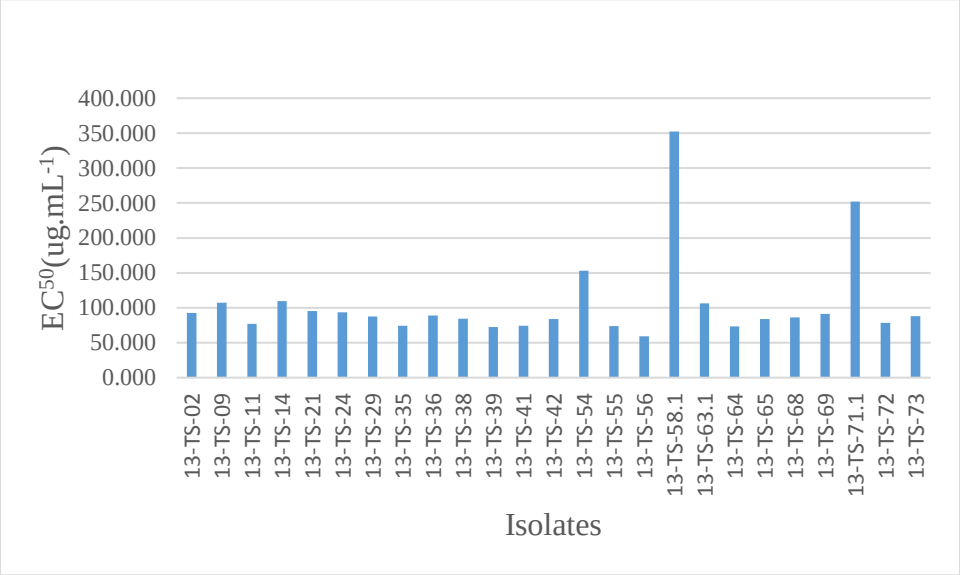
Appendix XXVII: Radial growth EC_{50} values for isolates of *Pyrenophora tritici-repentis* at Day 7 exposed to pyraclostrobin with SHAM from Saskatchewan in 2014.



Appendix XXVIII: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Saskatchewan in 2013.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
13-TS-02	Propiconazole	92.743
13-TS-09	Propiconazole	107.117
13-TS-11	Propiconazole	77.233
13-TS-14	Propiconazole	109.691
13-TS-21	Propiconazole	95.567
13-TS-24	Propiconazole	93.389
13-TS-29	Propiconazole	87.534
13-TS-35	Propiconazole	74.116
13-TS-36	Propiconazole	88.847
13-TS-38	Propiconazole	84.342
13-TS-39	Propiconazole	72.311
13-TS-41	Propiconazole	74.299
13-TS-42	Propiconazole	83.772
13-TS-54	Propiconazole	153.220
13-TS-55	Propiconazole	73.632
13-TS-56	Propiconazole	59.361
13-TS-58.1	Propiconazole	352.330
13-TS-63.1	Propiconazole	106.211
13-TS-64	Propiconazole	73.289
13-TS-65	Propiconazole	83.952
13-TS-68	Propiconazole	86.389
13-TS-69	Propiconazole	91.344
13-TS-71.1	Propiconazole	252.112
13-TS-72	Propiconazole	78.629
13-TS-73	Propiconazole	87.996

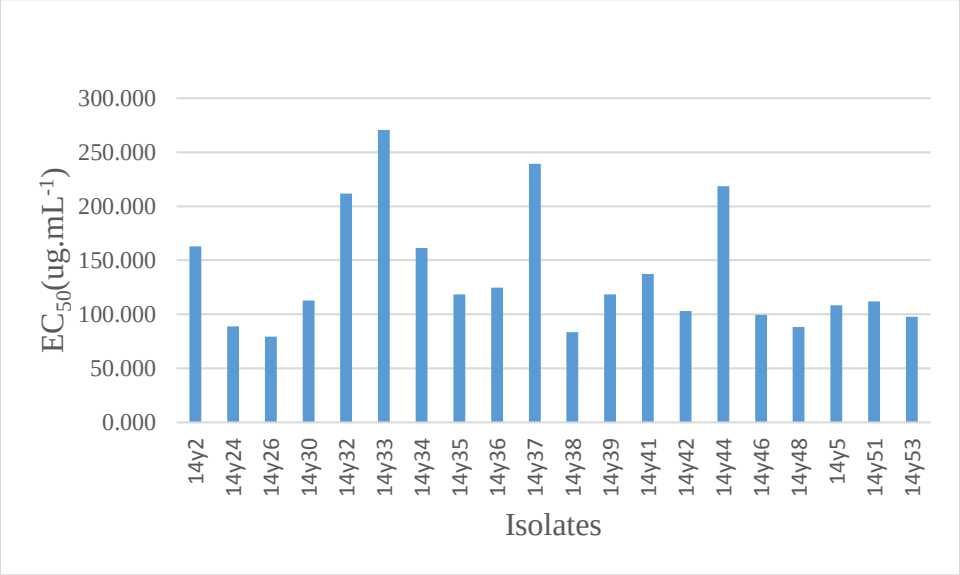
Appendix XXIX: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Saskatchewan in 2013.



Appendix XXX: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Saskatchewan in 2014.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
14y16	Propiconazole	101.000
14y2	Propiconazole	162.837
14y24	Propiconazole	88.721
14y26	Propiconazole	79.269
14y30	Propiconazole	112.846
14y32	Propiconazole	211.793
14y33	Propiconazole	270.523
14y34	Propiconazole	161.322
14y35	Propiconazole	118.387
14y36	Propiconazole	124.789
14y37	Propiconazole	239.388
14y38	Propiconazole	83.658
14y39	Propiconazole	118.552
14y41	Propiconazole	137.493
14y42	Propiconazole	103.004
14y44	Propiconazole	218.610
14y46	Propiconazole	99.401
14y48	Propiconazole	88.166
14y5	Propiconazole	108.260
14y51	Propiconazole	112.083
14y53	Propiconazole	97.808

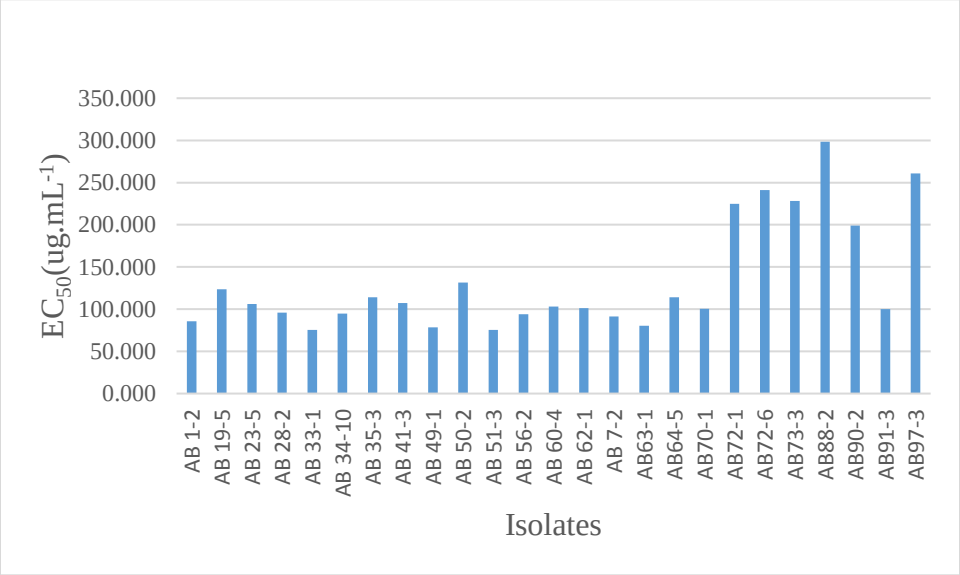
Appendix XXXI: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Saskatchewan in 2014.



Appendix XXXII: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Alberta in 2010.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
AB 1-2	Propiconazole	85.557
AB 19-5	Propiconazole	123.675
AB 23-5	Propiconazole	106.309
AB 28-2	Propiconazole	95.857
AB 33-1	Propiconazole	75.531
AB 34-10	Propiconazole	94.594
AB 35-3	Propiconazole	114.265
AB 41-3	Propiconazole	107.093
AB 49-1	Propiconazole	78.483
AB 50-2	Propiconazole	131.388
AB 51-3	Propiconazole	75.467
AB 56-2	Propiconazole	93.841
AB 60-4	Propiconazole	102.952
AB 62-1	Propiconazole	101.269
AB 7-2	Propiconazole	91.356
AB63-1	Propiconazole	80.392
AB64-5	Propiconazole	113.990
AB70-1	Propiconazole	100.363
AB72-1	Propiconazole	224.881
AB72-6	Propiconazole	241.034
AB73-3	Propiconazole	228.095
AB88-2	Propiconazole	298.309
AB90-2	Propiconazole	199.013
AB91-3	Propiconazole	100.013
AB97-3	Propiconazole	261.006

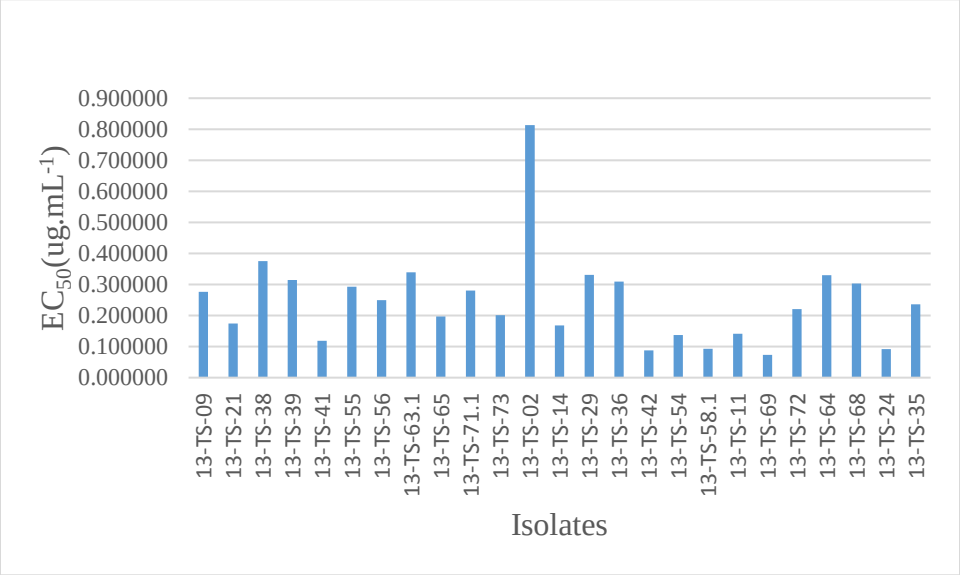
Appendix XXXIII: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to propiconazole from Alberta in 2010.



Appendix XXXIV: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Saskatchewan in 2013.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
13-TS-09	Pyraclostrobin	0.276100
13-TS-21	Pyraclostrobin	0.173900
13-TS-38	Pyraclostrobin	0.375200
13-TS-39	Pyraclostrobin	0.314700
13-TS-41	Pyraclostrobin	0.118400
13-TS-55	Pyraclostrobin	0.293400
13-TS-56	Pyraclostrobin	0.249100
13-TS-63.1	Pyraclostrobin	0.339200
13-TS-65	Pyraclostrobin	0.196600
13-TS-71.1	Pyraclostrobin	0.280500
13-TS-73	Pyraclostrobin	0.201100
13-TS-02	Pyraclostrobin	0.813400
13-TS-14	Pyraclostrobin	0.168500
13-TS-29	Pyraclostrobin	0.330600
13-TS-36	Pyraclostrobin	0.309700
13-TS-42	Pyraclostrobin	0.087450
13-TS-54	Pyraclostrobin	0.137400
13-TS-58.1	Pyraclostrobin	0.092700
13-TS-11	Pyraclostrobin	0.140900
13-TS-69	Pyraclostrobin	0.073400
13-TS-72	Pyraclostrobin	0.220800
13-TS-64	Pyraclostrobin	0.329800
13-TS-68	Pyraclostrobin	0.303400
13-TS-24	Pyraclostrobin	0.092300
13-TS-35	Pyraclostrobin	0.236100

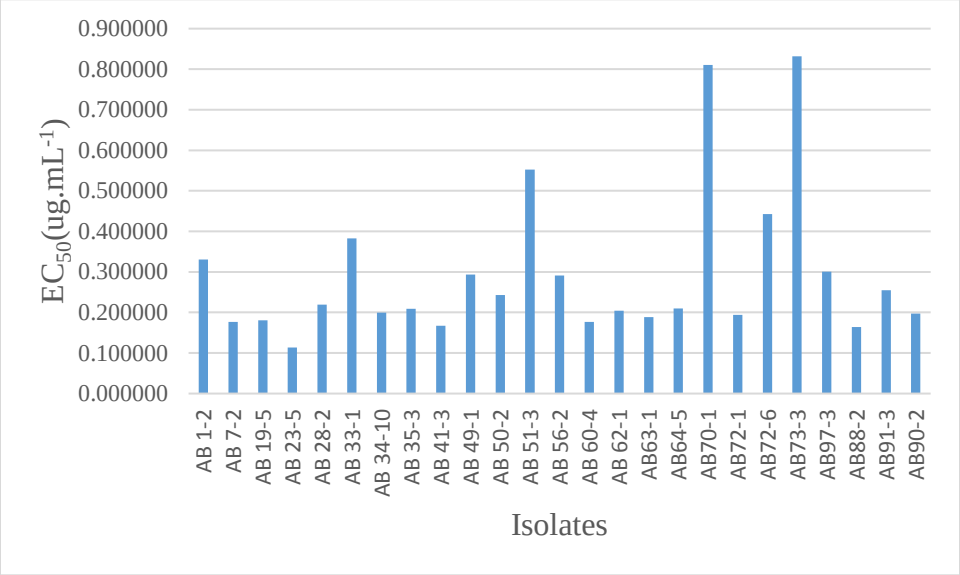
Appendix XXXV: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Saskatchewan in 2013.



Appendix XXXVI: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Alberta in 2010.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
AB 1-2	Pyraclostrobin	0.330500
AB 7-2	Pyraclostrobin	0.176500
AB 19-5	Pyraclostrobin	0.180300
AB 23-5	Pyraclostrobin	0.113900
AB 28-2	Pyraclostrobin	0.219400
AB 33-1	Pyraclostrobin	0.382400
AB 34-10	Pyraclostrobin	0.199300
AB 35-3	Pyraclostrobin	0.209200
AB 41-3	Pyraclostrobin	0.167200
AB 49-1	Pyraclostrobin	0.293400
AB 50-2	Pyraclostrobin	0.243200
AB 51-3	Pyraclostrobin	0.552300
AB 56-2	Pyraclostrobin	0.291300
AB 60-4	Pyraclostrobin	0.177000
AB 62-1	Pyraclostrobin	0.204000
AB63-1	Pyraclostrobin	0.188400
AB64-5	Pyraclostrobin	0.209900
AB70-1	Pyraclostrobin	0.810100
AB72-1	Pyraclostrobin	0.193700
AB72-6	Pyraclostrobin	0.443000
AB73-3	Pyraclostrobin	0.831600
AB97-3	Pyraclostrobin	0.300700
AB88-2	Pyraclostrobin	0.163700
AB91-3	Pyraclostrobin	0.255100
AB90-2	Pyraclostrobin	0.197400

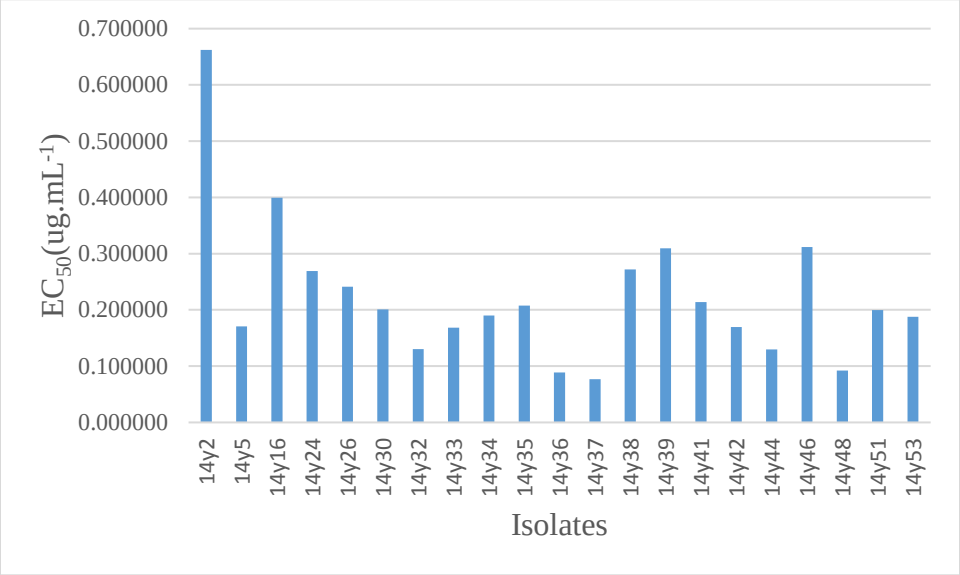
Appendix XXXVII: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Alberta in 2010.



Appendix XXXVIII: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Saskatchewan in 2014.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
14y2	Pyraclostrobin	0.662100
14y5	Pyraclostrobin	0.170900
14y16	Pyraclostrobin	0.399200
14y24	Pyraclostrobin	0.268900
14y26	Pyraclostrobin	0.241000
14y30	Pyraclostrobin	0.200700
14y32	Pyraclostrobin	0.130500
14y33	Pyraclostrobin	0.168300
14y34	Pyraclostrobin	0.189900
14y35	Pyraclostrobin	0.207400
14y36	Pyraclostrobin	0.089000
14y37	Pyraclostrobin	0.076600
14y38	Pyraclostrobin	0.272200
14y39	Pyraclostrobin	0.309600
14y41	Pyraclostrobin	0.214000
14y42	Pyraclostrobin	0.169300
14y44	Pyraclostrobin	0.129600
14y46	Pyraclostrobin	0.311800
14y48	Pyraclostrobin	0.092100
14y51	Pyraclostrobin	0.199900
14y53	Pyraclostrobin	0.187600

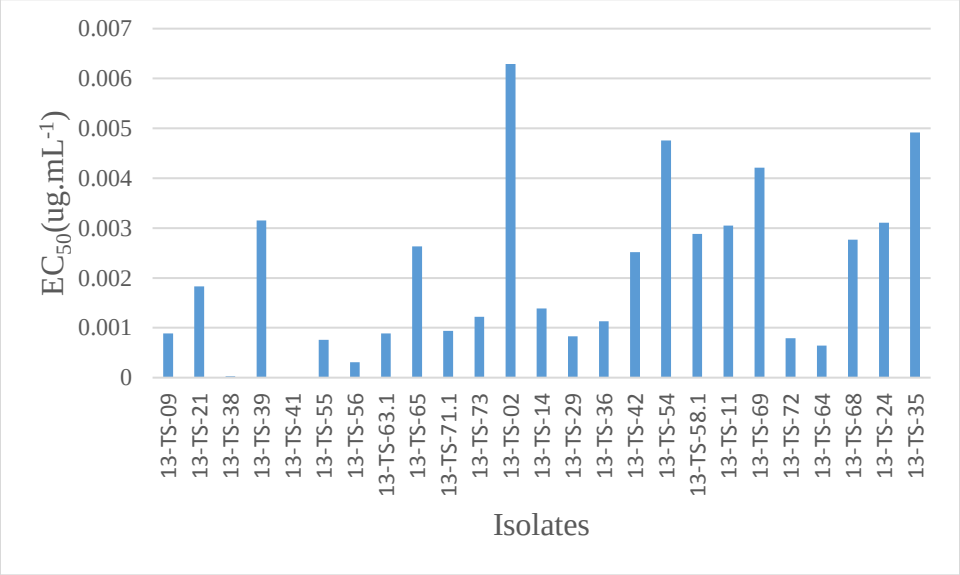
Appendix XXXIX: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin from Saskatchewan in 2014.



Appendix XL: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Saskatchewan in 2013.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
13-TS-09	Pyr+SHAM	0.00089
13-TS-21	Pyr+SHAM	0.00183
13-TS-38	Pyr+SHAM	0.00003
13-TS-39	Pyr+SHAM	0.00315
13-TS-41	Pyr+SHAM	0.00001
13-TS-55	Pyr+SHAM	0.00076
13-TS-56	Pyr+SHAM	0.00031
13-TS-63.1	Pyr+SHAM	0.00089
13-TS-65	Pyr+SHAM	0.00263
13-TS-71.1	Pyr+SHAM	0.00094
13-TS-73	Pyr+SHAM	0.00122
13-TS-02	Pyr+SHAM	0.00629
13-TS-14	Pyr+SHAM	0.00139
13-TS-29	Pyr+SHAM	0.00083
13-TS-36	Pyr+SHAM	0.00113
13-TS-42	Pyr+SHAM	0.00252
13-TS-54	Pyr+SHAM	0.00476
13-TS-58.1	Pyr+SHAM	0.00288
13-TS-11	Pyr+SHAM	0.00305
13-TS-69	Pyr+SHAM	0.00421
13-TS-72	Pyr+SHAM	0.00079
13-TS-64	Pyr+SHAM	0.00064
13-TS-68	Pyr+SHAM	0.00277
13-TS-24	Pyr+SHAM	0.00311
13-TS-35	Pyr+SHAM	0.00492

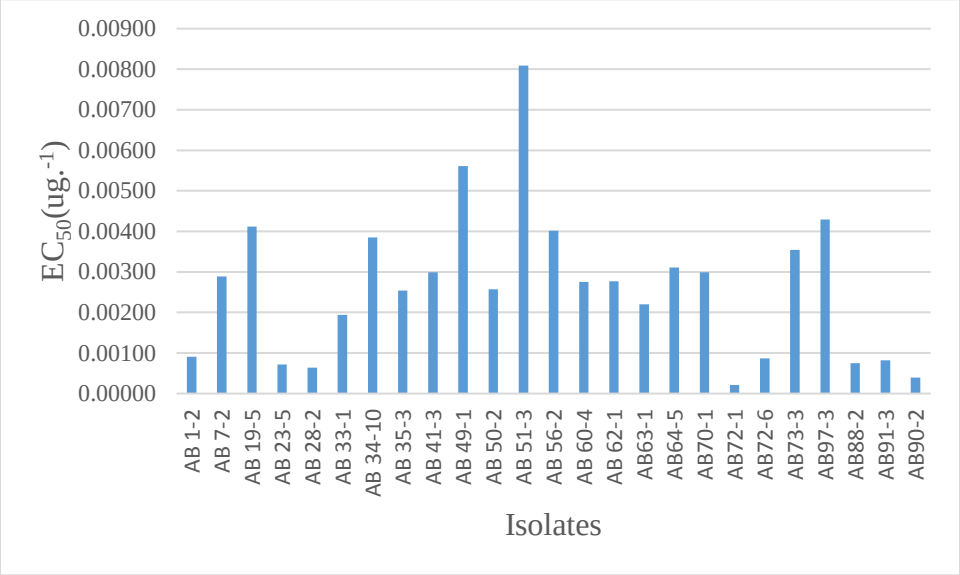
Appendix XLI: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Saskatchewan in 2013.



Appendix XLII: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Alberta in 2010.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
AB 1-2	Pyr+SHAM	0.00091
AB 7-2	Pyr+SHAM	0.00289
AB 19-5	Pyr+SHAM	0.00412
AB 23-5	Pyr+SHAM	0.00072
AB 28-2	Pyr+SHAM	0.00064
AB 33-1	Pyr+SHAM	0.00194
AB 34-10	Pyr+SHAM	0.00385
AB 35-3	Pyr+SHAM	0.00254
AB 41-3	Pyr+SHAM	0.00299
AB 49-1	Pyr+SHAM	0.00561
AB 50-2	Pyr+SHAM	0.00257
AB 51-3	Pyr+SHAM	0.00809
AB 56-2	Pyr+SHAM	0.00402
AB 60-4	Pyr+SHAM	0.00275
AB 62-1	Pyr+SHAM	0.00277
AB63-1	Pyr+SHAM	0.00220
AB64-5	Pyr+SHAM	0.00311
AB70-1	Pyr+SHAM	0.00299
AB72-1	Pyr+SHAM	0.00021
AB72-6	Pyr+SHAM	0.00087
AB73-3	Pyr+SHAM	0.00354
AB97-3	Pyr+SHAM	0.00429
AB88-2	Pyr+SHAM	0.00075
AB91-3	Pyr+SHAM	0.00082
AB90-2	Pyr+SHAM	0.00039

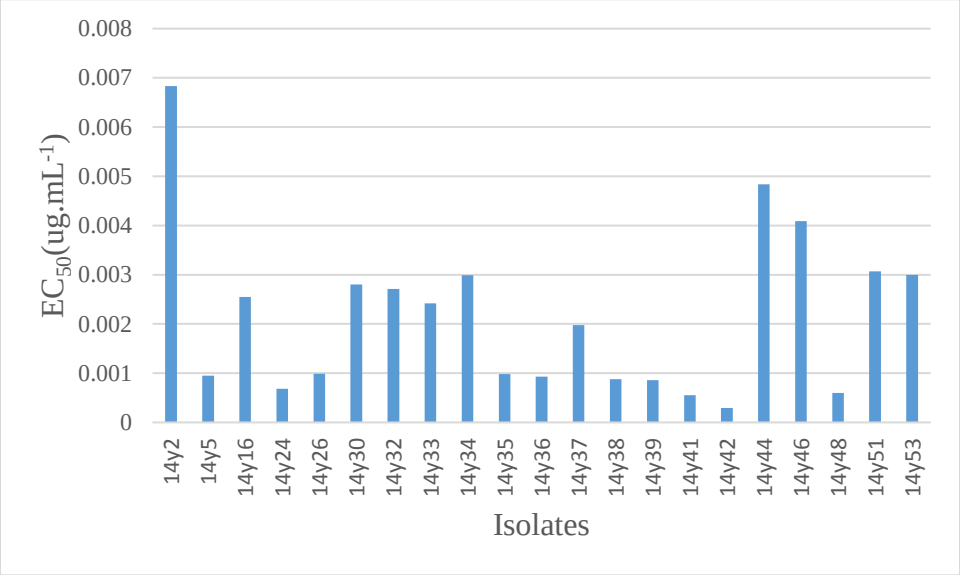
Appendix XLIII: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Alberta in 2010.



Appendix XLIV: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Saskatchewan in 2014.

Isolate	Fungicide	EC_{50} (µg mL^{-1})
14y2	Pyr+SHAM	0.00683
14y5	Pyr+SHAM	0.00095
14y16	Pyr+SHAM	0.00255
14y24	Pyr+SHAM	0.00068
14y26	Pyr+SHAM	0.00099
14y30	Pyr+SHAM	0.00280
14y32	Pyr+SHAM	0.00271
14y33	Pyr+SHAM	0.00242
14y34	Pyr+SHAM	0.00299
14y35	Pyr+SHAM	0.00098
14y36	Pyr+SHAM	0.00093
14y37	Pyr+SHAM	0.00198
14y38	Pyr+SHAM	0.00088
14y39	Pyr+SHAM	0.00086
14y41	Pyr+SHAM	0.00055
14y42	Pyr+SHAM	0.00029
14y44	Pyr+SHAM	0.00484
14y46	Pyr+SHAM	0.00409
14y48	Pyr+SHAM	0.00060
14y51	Pyr+SHAM	0.00307
14y53	Pyr+SHAM	0.00300

Appendix XLV: Germination EC_{50} values for isolates of *Pyrenophora tritici-repentis* exposed to pyraclostrobin with SHAM from Saskatchewan in 2014.



Appendix XLVI: ANOVA of yield (kg ha^{-1}) at low disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	405	0.76	0.7185

Appendix XLVII: ANOVA of final leaf disease (%) at low disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	285	15.99	<.0001

Appendix XLVIII: ANOVA of fusarium head blight (%) at low disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	111	1.02	0.4368

Appendix L: ANOVA of test weight (kg hL^{-1}) at low disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	345	0.98	0.4776

Appendix LI: ANOVA of thousand kernel weight (g) at low disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	404	1.06	0.3908

Appendix LII: ANOVA of protein content (%) at low disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	165	1.05	0.4068

Appendix LIII: ANOVA of yield (%) at high diseases locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	165	5.23	<.0001

Appendix LIV: ANOVA of final leaf disease (%) at high disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	165	30.95	<.0001

Appendix LV: ANOVA of fusarium head blight (%) at high disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	165	0.75	0.7296

Appendix LVI: ANOVA of test weight (kg hL^{-1}) at high disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	165	4.31	<.0001

Appendix LVII: ANOVA of thousand kernel weight (g) at high disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	165	15.88	<.0001

Appendix LVIII: ANOVA of protein content (%) at high disease locations.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	45	1.11	0.3714

Appendix LIX: ANOVA of yield (%) at Melfort 2013.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	345	0.73	0.7558

Appendix LXX: ANOVA of final leaf disease (%) at Melfort 2013.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	225	9.73	<.0001

Appendix LX: ANOVA of fusarium head blight (%) at Melfort 2013.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	344	0.9	0.5656

Appendix LXI: ANOVA of test weight (kg hL^{-1}) at Melfort 2013.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	285	0.95	0.5136

Appendix LXII: ANOVA of thousand kernel weight (g) at Melfort 2013.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	344	0.91	0.5478

Appendix LXIII: ANOVA of protein content (%) at Melfort 2013.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
trt	15	105	1.21	0.2788

Appendix LXIV: ANOVA of propiconazole spore germination EC_{50} of *Pyrenophora tritici-repentis.*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	823654	11937	17.78	<.0001
Error	140	93979.7	671.283		
Corrected Total	209	917634			

Appendix LXV: ANOVA of pyraclostrobin spore germination EC_{50} of *Pyrenophora tritici-repentis.*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	70	5.26872	0.07527	13.61	<.0001
Error	142	0.7855	0.00553		
Corrected Total	212	6.05422			

Appendix LXVI: ANOVA of pyraclostrobin with SHAM spore germination EC_{50} of *Pyrenophora tritici-repentis.*

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	70	0.00062	8.9E-06	4.21	<.0001
Error	142	0.0003	2.1E-06		
Corrected Total	212	0.00092			

Appendix LXVII: ANOVA of propiconazole radial growth EC_{50} of *Pyrenophora tritici-repentis* at Day 4.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	1.26381	0.01832	1.64	0.0071
Error	138	1.53855	0.01115		
Corrected Total	207	2.80236			

Appendix LXVIII: ANOVA of propiconazole radial growth EC_{50} of *Pyrenophora tritici-repentis* at Day 7.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	8.60191	0.12467	1.47	0.0295
Error	139	11.8256	0.08508		
Corrected Total	208	20.4275			

Appendix LXIX: ANOVA of pyraclostrobin radial growth EC_{50} of *Pyrenophora tritici-repentis* at Day 4.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	23.6278	0.34243	4.2	<.0001
Error	134	10.9198	0.08149		
Corrected Total	203	34.5476			

Appendix LXX: ANOVA of pyraclostrobin radial growth EC_{50} of *Pyrenophora tritici-repentis* at Day 7.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	48.5973	0.70431	1.64	0.0075
Error	138	59.4193	0.43057		
Corrected Total	207	108.017			

Appendix LXXI: ANOVA of pyrclostrobin with SHAM radial growth EC_{50} of *Pyrenophora tritici-repentis* at Day 4.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	0.0437	0.00063	3.01	<.0001
Error	137	0.02881	0.00021		
Corrected Total	206	0.0725			

Appendix LXXII: ANOVA of pyraclostrobin with SHAM radial growth EC_{50} of *Pyrenophora tritici-repentis* at Day 7.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	69	0.14454	0.00209	4.49	<.0001
Error	138	0.06437	0.00047		
Corrected Total	207	0.2089			